Influence of natural enemies on *Cirsium arvense* — a biogeographic perspective

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

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Cirsium arvense (L.) Scop. (Californian, Canada, or creeping thistle) is an exotic perennial herb indigenous to Eurasia that successfully established in New Zealand (NZ) approximately 130 years ago. Presently, *C. arvense* is considered one of the worst invasive weeds in NZ arable and pastoral productions systems. The mechanism most commonly invoked to explain the apparent increased vigour of introduced weeds is release from natural enemies. The enemy release hypothesis (ERH) predicts that plants in an introduced range should experience reduced herbivory, particularly from specialists, and that release from this natural enemy pressure facilitates increased plant performance in the introduced range. In 2007 broad surveys were carried out in NZ and central Europe in order to quantify and compare growth characteristics of *C. arvense* in its native vs. introduced range. Additionally, permanent field plots were established in NZ and Europe where natural enemies were excluded with the use of insecticide and fungicide applications, and compared with controls (ambient natural enemy pressure). The impact of the specialist leaf-feeding beetle, *Cassida rubiginosa* Müller, which was recently released in NZ as a biological control agent against thistles, was also assessed.

From the field surveys, significantly more endophagous herbivory was present in the native range compared to the introduced range, as predicted by the ERH. Endophagous herbivory in NZ was solely from the capitulum-feeding weevil, *Rhinocyllus conicus* (Frölich), and was only found in the North Island surveys. No stem mining attack was found anywhere in NZ. The proportion of shoots attacked by the specialised rust pathogen, *Puccinia punctiformis* (Str.) Röhl., was similar in both the native and introduced ranges. Interestingly, this has casted doubt on the idea that stem-mining vectors, such as *Ceratapion onopordi* Kirby, are important for transmission of the rust

pathogen. Contrary to the ERH, there were no significant difference in plant performance between the native and introduced ranges, or differences could be explained by simple climatic factors. Climate tended to be more favourable for growth of *C. arvense* in NZ. In the permanent field plots in the native range, population growth of *C. arvense* was significantly greater where natural enemies were excluded, suggesting that insect herbivores and pathogens might have a regulating influence on the population growth of this plant. Furthermore, the probability of shoots transitioning to the reproductive growth stage was enhanced when insect herbivores were excluded, indicating that natural enemies might influence plant development. The biological control agent *C. rubiginosa* reduced the growth of *C. arvense*, although the impact of this herbivore was minimal in comparison to interspecific plant competition. Thus, although there is reduced specialist natural enemy pressure in NZ, the growth of *C. arvense* is not significantly different from in its native range. Nevertheless, there is some evidence that natural enemies in the native range might have a regulating influence on the population dynamics of the plant, and that the specialist herbivore, *C. rubiginosa*, can impact the plant in certain conditions.

Keywords: *Cirsium arvense*, Californian thistle, Canada thistle, creeping thistle, *Cassida rubiginosa*, *Ceratapion onopordi*, *Rhinocyllus conicus*, *Puccinia punctiformis*, herbivory, biological control, invasive species, enemy release hypothesis.

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Chapter 1

Introduction

Invasive plants often reach higher densities and biomasses per unit area, persist longer, and occupy a wider set of environmental conditions compared with conspecifics in their native range (Crawley 1987; Hinz & Schwarzlaender 2004). One of the most common explanations for the increased vigour of invasive plants is the release from natural enemies (Elton 1958; Maron & Vilà 2001; Keane & Crawley 2002; Mitchell & Power 2003). The enemy release hypothesis (ERH) asserts that after introduction to an exotic region, plants experience a reduction in natural enemy pressure, which facilitates their increased dispersal and abundance. The hypothesis is based on two assumptions: 1) plants experience reduced natural enemy pressure, particularly from specialists when introduced into a new range, and 2) natural enemies are able to regulate plant populations.

Several studies have shown that introduced plants often experience decreased herbivore damage compared with conspecifics in their native range, supporting the first assumption of the enemy release hypothesis (ERH) (e.g. Memmott *et al.* 2000; Wolfe 2002; Genton *et al.* 2005). However, the fact that herbivores damage plants does not indicate that they are capable of regulating plant population dynamics (Crawley 1989). Studies investigating the impact of natural enemies on plant population dynamics have yielded mixed results with outcome appearing to depend on the life history of the plant, the herbivore involved, and environmental conditions (Louda 1982, 1994; Root 1996; Edwards *et al.* 2000; Maron & Gardner 2000).

Classical biological control of weeds has a long history of success (Debach & Rosen 1991; Fowler *et al.* 2000), but also many failures (Julien & Griffiths 1998). The successes suggest that, at least in some cases, natural enemies are capable of regulating plant populations. In fact, the assumed maxim of the ERH, based on its intrinsic merit, has been the premise of many classical biological control programs (Debach & Rosen 1991). Successful classical biological control lends pertinent evidence in support of the ERH, but is by no means a test of the hypothesis (Keane & Crawley 2002). A more direct test of the ERH would include manipulative, experimental exclosure studies in both the native and exotic ranges of an invasive plant. The importance of this type of research for the identification of invasion mechanisms has been emphasized in several reviews (Crawley 1989; Maron & Vilà 2001; Hinz & Schwarzlaender 2004; Hierro *et al.* 2005; Liu & Stiling 2006).

As part of the ongoing classical biological control program against *Cirsium arvense* (L.) Scop. in New Zealand (NZ), biogeographical studies were conducted to compare plant performance between the native and introduced ranges, and to investigate the role of natural enemies in regulating the population dynamics of this weed. *C. arvense* is a perennial herb indigenous to Eurasia, and was first reported as introduced to NZ in 1878 (Kirk 1878). It is one of the most problematic weeds in NZ, casuing severe economic losses (Hartley & James 1979; Bourdôt *et al.* 2007). Recently, renewed interest in biological control of *C. arvense* in NZ has resulted in the release of the stem-ming weevil, *Ceratapion onopordi* Kirby, and the leaf-feeding beetle, *Cassida rubiginosa* Müller.

The purpose of the present research was to assess the influence of natural enemies (insect herbivores and pathogens) on *C. arvense* as part of the biological control effort against this weed in NZ, and to test the ERH in relation to *C. arvense*. This research consisted of three primary aspects. Firstly, to conduct comparative field surveys in the native (Europe) vs. introduced (NZ) ranges in order to assess the natural enemy pressure in both ranges and to test the commonly perceived notion that *C. arvense* is more vigorous in its introduced range. Secondly, since many factors might account for differences in growth patterns of the plant between ranges, natural enemy exclosure experiments were set up in both the native and introduced range in order to more specifically test the ERH. Thirdly, the impact of the recently released biocontrol agent, *C. rubiginosa*, was assessed in an interspecific plant competition experiment, and in an open field release experiment in Europe. This research has increased our understanding of the importance of natural enemies for regulating this plant, and has demonstrated that valuable information can be gained by conducting biogeographcal studies on invasive weeds in their native and introduced ranges. Furthermore, this research has advanced our understanding of multi-trophic interactions of natural enemies for improving biological control of weeds.

In this thesis the following hypotheses were tested:

Chapter 3

(H1) Performance of *C. arvense* would be greater in the introduced ranges of the North and South Islands of New Zealand.

(H2) Herbivory on *C. arvense* would be greater in the native range.

Chapter 4

(H1) Does transmission of the rust pathogen, *Puccinia punctiformis*, require stem mining vectors?

Chapter 5

(H1) In the native range, exclusion of insects or pathogens would have a positive effect, and that dual exclusion of insects and pathogens would have an increased positive effect on shoot performance, development, and population growth, relative to controls (ambient natural enemy pressure).

(H2) In the introduced range, exclusion of insects or pathogens and dual exclusion of both would not change shoot performance, development, or plant population growth, relative to controls.

Chapter 6

(H1) The different simulated grazing treatments would alter the competitive balance so that ungrazed treatments would reduce *C. arvense* performance more than grazed treatments and long-grazed treatments would reduce *C. arvense* performance more than short-grazed treatments.

(H2) Herbivory by *Cassida rubiginosa* would reduce the performance of *C. arvense*, and that high larval densities would have a greater effect than low densities.

(H3) The interaction of competition and *C. rubiginosa* would be additive, with both factors contributing to decrease the performance of *C. arvense*.

(H4) A realistic outbreak density of *C. rubiginosa* larvae would reduce the growth and development of *C. arvense* in an open field situation.

Chapter 7

General discussion of the combined results and future reseach needs.

Chapter 2

Literature Review

2.1 STUDY SYSTEM

2.1.1 Taxonomy and nomenclature

Cirsium arvense (L.) Scop. belongs to the family Asteraceae (=Compositae), and is a member of the tribe Cardueae, which comprises the plants commonly known as thistles (Bremer 1994). Detmers (1927) noted that the name *Cirsium arvense* was first used by Tourefort in 1700. However, in general, pre-Linnean authorities should be avoided. Linneaus originally recorded the plant as *Serratula arvensis*, which was subsequently reclassified as *Cirsium arvense* by Scopoli in 1772, which revived an early pre-Linnean name, but nonetheless conformed to the tradition of binomial nomenclature (see Detmers, 1927). Other erroneous synonyms appearing in the literature include *Carduus arvensis* and *Cnicus arvensis* (Webb *et al.* 1988). Moore (1975) recoginized four varieties of *C. arvense* in North America: *C. arvense* var. *vestitum*, *C. arvense* var. *integrifolium*, *C. arvense* var. *arvense* and *C. arvense* var. *horridum*.

Several common English names exist for *C. arvense,* of which the most notable are creeping thistle, Canada thistle, and Californian thistle. The common names Canada and Californian thistle reflect what is assumed to be the source of importation in the U.S. and Australia. The common name Canada thistle likely arose from the notion that it was first introduced to eastern Canada by early European colonists in the late 1600's, and from there spread to the U.S. (Hodgson 1968). The name Californian thistle is thought to be the result of *C. arvense* seed first brought to Tasmania as impurity in a shipment of oats from California (Holland 1931). The original source of *C. arvense* importation into New Zealand is unknown, but the Australian common name was adopted.

2.1.2 Plant description

The morphological description of *C. arvense* is fairly standard throughout the literature (for more detailed descriptions see Detmers, 1927; Moore, 1975; Webb *et al.* 1988). *C. arvense* is a perennial herb that spreads clonally by means of creeping lateral roots. The height and leaf shape of *C. arvense* exhibits extreme phenotypic variation. The height of flowering shoots varies from 0.3 to 1.5 m. Leaves are alternate and sessile and vary in texture, vestiture, segmentation and spininess, which are the basis for classification of

different varieties in North America (Moore 1975). The flowers of *C. arvense* are almost completely dioecious (pistals and stamens on different plants). Numerous flower heads are produced on each plant shoot with purple to pinkish florets. Male flower heads are globular and slightly smaller than the flask-shaped female heads (Moore 1975). Seeds are produced in an achene attached to a pappus. *C. arvense* is a long-day plant requiring a 14 to 16 hour photoperiod to induce flowering. In New Zealand, *C. arvense* flowers from December to February, and seeds are produced from December to April (Webb *et al.* 1988). The creeping lateral roots and the dioecious nature of the plant are unique features of *C. arvense* in comparison with other thistles. *C. arvense* can also be distinguished from other thistles by the smooth spineless stems, numerous small spineless seedheads, and increased branching towards the top of the plant (Moore 1975). Several hybrids of *Cirsium* species are known from the native range in Europe (Bureš *et al.* 2004). Of the four species of *Cirisum* occurring in New Zealand (Webb *et al.* 1988), hybrids are known from Europe among *C. arvense*, *C. palustre* and *C. vulgare* (Detmers 1927; Moore 1975; Clapham *et al.* 1987). However, no hybrids have been reported in the literature from New Zealand.

2.1.3 Sexual reproduction

New infestations of *C. arvense* are often the result of seed disseminated by female (pistillate) plants. Seed production by *C. arvense* is dependent on insect pollination and proximity to male (staminate) plants. Population sex ratios range from equal male:female ratios (Lloyd & Myall 1976) to extremely female biased sex ratios (Lalonde & Roitberg 1994). In general, few seeds are produced where male and female plants are separated by more than 50 metres (Lalonde & Roitberg 1994); however Amor & Harris (1974) reported some seeds being formed where the nearest males were a distance of 390 metres away. Single shoots can produce up to 100 seedheads, each containing approximately 75 seeds (Detmers 1927; Moore 1975). Mature seeds are disseminated 2-3 weeks after pollination (Lalonde & Roitberg 1989). Generally the achene-pappus units are not carried far from the parent plant, but may occasionally be carried long distances (i.e.1km) under favourable environmental conditions (Bakker 1960; Sheldon & Burrows 1973). Bakker (1960) also noted that the pappus quite readily breaks off from the achene, thereby frequently restricting long-distance dispersal.

However, other than wind dispersal, seeds can be transported by machinery, contaminated crop seed, manure, and irrigation water. Seed germination was reported to

vary from 52 to 97% at fields in Victoria, Australia (Amor & Harris 1974). Seed survival in the soil is also variable, but increases with burial depth. Seeds buried more than 0.2 m deep have been reported to germinate after 20 years. However, typically seeds are not buried so deeply, or are simply left on the ground surface, where they either germinate or decay (Donald 1994). Therefore, managing seed bank dynamics is unlikely to be an important factor for controlling this weed. Furthermore, in established populations of this weed, recruitment from seedlings is rare (Edwards *et al.* 2000; Bourdôt *et al.* 2006b), and therefore most management effort is focused on controlling vegetative growth and reproduction.

2.1.4 Vegetative reproduction

Vegetative growth and reproduction are important for establishment and colonization.

C. arvense spreads clonally by means of creeping lateral roots [sometimes incorrectly referred to as rhizomes, (see Donald 1994)] from which an average of eight adventitious shoots arise per metre of root (Nadeau & Vanden Born 1989). Adventitious shoots have been reported to emerge from root fragments buried 0.5 m deep (Hamdoun 1972). Detmers (1927) noted that lateral roots are often at a depth greater than reached by typical tillage. This was confirmed by a detailed study of the root system of *C. arvense* carried out by Nadeau & Vanden Born (1989) in Alberta, Canada. They found that approximately one third of the total root biomass was in the top 0.2 m of soil, another third in the 0.2-0.4 m depth, and the remaining third below 0.4 m, with roots extending as deep as 1.8 m. Maximum depths of *C. arvense* roots are variable depending on soil type, and have been reported to range from 1 to 6.75 m, with greater depths occurring in clay soils, in which the plant particularly thrives (Detmers 1927; Rogers 1928). New roots are produced each year by the new population of aerial shoots, and the clone spreads radially. However, individual roots do not persist for more than 2 years (Rogers 1928). This pattern of spread was reported by Amor & Harris (1975) in Australia where they documented increased density and shoot height towards the perimeter of the patch, indicating senescence of old rootstocks towards the centre. Studies in New Zealand have also indicated that a high proportion of root biomass does not survive in successive growing seasons (Bourdôt *et al.* 1998; Bourdôt *et al.* 2000a). The relatively short life-span of the roots means that over time a clone will natural fragment. Thus, in an established patch of *C. arvense*, genetic variability is likely to be low, but composed of

many individual units. The implications of this are that compensation for herbivory by mobilization of resources from more distant parts of the clone is unlikely.

2.1.5 Chemical composition

The major secondary chemical constituents of the Cardueae are similar to those found in the family Asteraceae. However, the Cardueae are noted to have a markedly higher percentage of lipophilic compounds (e.g. lipids and terpenoids) (Wagner 1977). Several flavanoid and alkaloid compounds have been identified from *C. arvense*. The flavanoids and alkaloids are well characterized as putative secondary defensive compounds against generalist herbivory (i.e. qualitative defences). Lipid deposition on *C. arvense* leaves varies significantly among ecotypes, and has been attributed to varying degrees of herbicide resistance (Hodgson 1973). Lipids could be considered quantitative defensive compounds (i.e. digestibility-reducing chemicals), which could contribute to specialist herbivore resistance. To date, the defensive chemistry of *C. arvense* in relation to specialist and generalist herbivory has not been studied.

2.1.6 Allelopathy

The allelopathic potential of *C. arvense* has been tested in Petri dish and greenhouse bioassays. Root and shoot extracts of *C. arvense* inhibited germination of several crops and weeds in laboratory experiments (Bendall 1975; Stachon & Zimdahl 1980). When *C. arvense* field litter was incorporated into potted plants in a greenhouse study the growth of *Amaranthus retroflexus, Setaria viridis*, and *Hordeum vulgare* was reduced compared with controls (Stachon & Zimdahl 1980). However, Fuerst & Putman (1983) suggested that a system analogous to Koch's postulates be applied to allelochemicals in order to conclusively demonstrate allelopathy. Therefore, although these studies indicate some potential for *C. arvense* to be allelopathic, they do not meet the minimum criteria necessary to conclusively demonstrate allelopathy.

2.1.7 Management

Cultural control

Early efforts at controlling *C. arvense* focused on regular tillage or mowing in an attempt to deplete the roots of energy for re-growth (Detmers 1927; Derscheid *et al.* 1961; Hodgson 1968). Mowing or tillage will eventually kill the plant, however this method is labour intensive, and requires 2 to 3 years to completely eliminate the plant (Donald

1990). Combining mowing with the use of a competitive forage crop has been noted to be an effective management strategy (Hodgson 1968). A forage crop such as *Medicago sativa*, which emerges in early spring, can effectively "smother" competing weeds. Cutting the forage for hay at least twice during the growing season can drastically reduce *C. arvense* populations (Hodgson 1968). Proper grazing management can also serve to reduce *C. arvense* infestations. Recently, De Bruijn & Bork (2006) showed that 2 to 3 years of high intensity grazing twice during the growing season significantly reduced *C. arvense* density in pastures. However, season long grazing maintained or increased the severity of *C. arvense* infestation.

Chemical control

Chemical control is the most widely studied aspect of *C. arvense*, and has been reviewed by Donald (1990). Extensive effort has been invested into chemical control of *C. arvense*, particularly in annual crop lands. Early attempts at chemical control used formulations of sodium arsenite and sodium chlorate, with limited success (Detmers 1927; Holland 1931). Many modern herbicides are now used for the control of *C. arvense* including 2,4-D, dicamba, picloram, metasulfuron, atrazine and glyphosate. Resistance of different *C. arvense* ecotypes to 2,4-D was noted as early as early as 1956 (Rasmussen 1956), and is now well documented (Hodgson 1958; 1964; 1970; 1973). Therefore, alternating among herbicides with different modes of action is recommended, to avoid selecting for resistant ecotypes (Donald 1990). Glyphosate is also a common herbicide used for control of *C. arvense*, and can be effective in non-cropping areas or fallow fields (Donald 1990). In New Zealand, autumn glyphosate application is recommended for control of *C. arvense* in pastures (D. Moot, personal communication). Herbicide application is generally most effective when applied at the budding stage of the plant, when carbohydrate reverses in the roots are lowest (Donald 1990). For satisfactory herbicide control, repeated applications over several years are usually necessary. In some cases repeated 2,4-D applications were no more effective than planting a competitive forage and mowing (Derscheid *et al.* 1961). Difficulties with chemical control of *C. arvense* are attributed to resistant ecotypes, large root reserves, and ecotype variation in plant phenology (Donald 1990).

Biological control

Classical biological control of *C. arvense* has been attempted in Canada, the U.S., and New Zealand (Julien & Griffiths 1998). Thus far, five insect species have been intentionally released for biocontrol of *C. arvense* (Table 3.1). Investigations into the potential for biological control of *C. arvense* in North America began in 1961 at the Commonwealth Institute of Biological Control, Switzerland (now CABI-Europe Switzerland). Numerous potential biocontrol agents were identified from European surveys that varied in their degree of host specificity (monophagous, oligophagous, or polyphagous) (Zwölfer 1965). The preliminary surveys carried out in western Europe by Zwolfer (1965) identified 86 phytophagous species commonly associated with *C. arvense*. Of these 86 insects 11 were considered monophagous or nearly so, 30 were oligophagous (only feeding on the subtribe Carduinae), and the rest polyphagous.

To date, the five insects released for control of *C. arvense* have either failed to establish (Table 2.1), or have had little impact on *C. arvense* populations. Accidental introductions of specialised insects of Eurasian origin have occurred in North America (Table 2.2). However, in New Zealand the only non-deliberate introduction of a potential biocontrol agent on *C. arvense* is the rust fungus, *Puccinia punctiformis*. *C. arvense* was the subject of New Zealand's first investigations into biological control of weeds using fungi (Johnston 1990). Both Cockayne (1914; 1915) and Cunningham (1927) undertook trials to augment infection of *P. punctiformis* on *C. arvense* populations in New Zealand but recorded minimal success due to the lack of systemic infection. Cunningham (1927) also first recorded the incidence of *Sclerotinia sclerotiorum* and a species of *Fusarium* causing disease on *C. arvense* and proposed both as potential agents for biological control. The non-host-specific fungal pathogen, *S. sclerotiorum*, has now been extensively studied as a potential mycoherbicide for *C. arvense* (Bourdôt *et al.* 1995; Bourdôt *et al.* 2000b; Bourdôt *et al.* 2006b). Studies by Graeme Bourdôt and colleagues have indicated that this pathogen can have a serious impact on *C. arvense* populations (Bourdôt *et al.* 2006b), and that the risk associated with spreading this pathogen is minimal (Bourdôt *et al.* 2006a). However, the fact that *S. sclerotiorum* is a common, and often serious disease of crops, is a likely reason for reluctance to utilize this pathogen as a biocontrol agent.

Other necrotrophic fungal diseases of *C. arvense* that have been investigated locally for their inundative biocontrol potential include; *Phoma exigua* var.e*xigua* (Bithell & Stewart 2001), *Verticillium dahliae* (Waipara *et al.* 1997), *Fusarium oxysporum,*

Septoria cirsii, Alternaria sp. and a bacterial shoot rot *Pseudomonas* sp. (Waipara *et al.* 1991). Popay & Cheah (1990) studied the potential of root pathogens for biocontrol of *C. arvense* using three bacterial species of *Erwinia*, *Pseudomonas*, and *Xanthomonas* and a root rot species of *Pythium*. *Albugo tragonoponis,* a biotrophic white rust pathogen, has also been reported to cause disease outbreaks on *C. arvense* in New Zealand (Pennycook 1989). Internationally, several pathogens have also been considered for use as bioherbicides against *C. arvense*. Bailey *et al.* (2000) isolated 287 pathogenic fungi and the bacterium *Pseudomonas syringe* pv. *tagetis* (PST) on *C. arvense* from western Canada. From this group of pathogens isolates of *Phoma*, *Phomopsis*, *Colletotrichum*, and *Fusarium* were found to be pathogenic, and had potential for incorporation into a bioherbicide forumlation.

Table 2.1. Insects deliberately released for biological control of *Cirsium arvense* in North America and New Zealand (Julien & Griffiths, 1998).

Species	**Release date(s)**	**Country**	**Feeding niche**	**Established**
Altica carduorum Guér.	1963 1966, 1970 1979, 1990	Canada USA New Zealand	exophagous leaf chewer	No No ?
Hadroplontus litura (F.) (=*Ceutorhynchus litura*)	1965 1971 1976, 1988	Canada USA New Zealand	endophagous stem-borer	Yes Yes ?
Lema cyanella (L.)	1978, 1983, 1994 1983, 1990	Canada New Zealand	exophagous leaf chewer	Yes Yes
Rhinocyllus conicus (Frölich)[†]	1968	Canada	endophagous flowerhead feeder	Yes
Urophora cardui (L.)	1974 1977 1976, 1995, 1996	Canada USA New Zealand	endophagous stem-galler	Yes Yes ?

[†] *R. conicus* was released in the USA and NZ for control of invasive *Carduus* spp., but will also attack *C. arvense*. It is established in NZ and has been found feeding on *C. arvense*.

Table 2.2. Accidental introduction of natural enemies of *Cirsium arvense* in North America and New Zealand (Julien & Griffiths 1998).

Species	Establishment date	Country	Feeding niche
Cassida rubiginosa Müller	pre-1901	Canada USA	Foliage feeder
Larinus planus (F.)	pre-1968	Canada USA	Seedhead feeder
Cleonus piger (Scop.)	pre-1933 pre-1929	Canada USA	Root crown miner
Dasyneura gibsoni Felt.	?	Canada	Flower galls
Terellia ruficauda F. (=*Orellia ruficauda*)	pre-1927	Canada	Seedhead feeder
Puccinia punctiformis (Str.) Röhl.	pre-1912	Canada USA New Zealand	Pathogen causing localised and systemic infection

2.1.8 Issues of non-target effects

In North America there are 92 native *Cirsium* species, some of which are considered threatened or endangered (Moore & Frankton 1974; USDA-NRCS 2006). In the 1990s evidence that the deliberately released biocontrol agent, *Rhinocyllus conicus,* was attacking rare and endangered *Cirsium* species native to North America drew attention to possible negative ecological consequences of biological control (Louda *et al.* 1997; Strong 1997; Louda 1998, 1999). The evidence of non-target effects of *R. conicus* stimulated serious re-evaluation of the host specificity of any future agents for classical weed biocontrol. However, it was evident from early host specificity testing that *R. conicus* could develop on other *Cirsium* species. Thus the science of biocontrol was appropriate, but in the decades since the release of *R. conicus*, societal and scientific viewpoints on biodiversity conservation have changed. The issues with non-target effects of *R. conicus* in North America may have delayed progress in weed biocontrol in New Zealand. However, it is evident that New Zealand is in a unique situation with regard to biocontrol of thistles. In New Zealand there are no native species in the tribe Cardueae, and the closest related species that are native to New Zealand belong to the tribe Lactuceae. The absence of native thistle species in New Zealand provides a unique opportunity for biological control, since the many oligophagous (Cardueae specialists) species in Europe can be considered for classical biocontrol.

2.2.9 Biocontrol agents of particular interest

Puccinia punctiformis (Str.) Röhl. (Uredinales: Pucciniaceae) (=*P. obtegens*, *P. suaveolens*) exists in all regions of the world where *C. arvense* is present. It has never been intentionally released for classical biological control, but its importance as a potential biocontrol agent has been long recognized in New Zealand (Cockayne 1915). It is an autoecious rust fungus completing its entire life cycle on *C. arvense* (Fig. 3.1). *P. punctiformis* is a macrocylic rust fungus, producing all five possible spore stages (teliospores, basidiospores, spermatia, aeciospores, and uredospores). To date only three of the five spore stages, spermagonia, uredospores and teliospores have been recorded on *C. arvense* plants in New Zealand (Cunningham 1931; N. Waipara, personal observation). Of the five spore stages of the fungus, basidiospores, aeciospores, and uredospores cause infection. The teliospores are the sexual and overwintering stage, which upon germination give rise to basidium and basidiospores. Upon infection from basidiospores, spermagonia are formed, which contain spermatia. Spermatia act as male gametes, and function to fertilize receptive hyphae of compatible mating types. The resultant mycelium gives rise to aecia containing aeciospores. Aeciospores, upon infection give rise to uredia containing uredospores. At plant maturity, infection from uredospores results in production of telia and teliospores. Although most *Puccinia* fungi are believed to be commonly dispersed by windblown spores (Agrios 2005), Friedli & Bacher (2001b) suggest that insect vectors are critical for the dispersal of *P. punctiformis*.

Systemic infection is initiated by basidiospores produced from germinating teliospores. Systemic infection by *P. punctiformis* has severe detrimental effects on *C. arvense*, often killing developing shoots (Watson & Keogh 1980; Thomas *et al.* 1994). Understanding the mechanism of teliospore germination is seen as a key step to enhance biological control with this fungus. Volatiles compounds from the roots and seeds of *C. arvense* were found to stimulate *P. punctiformis* teliospore germination (French *et al.* 1988; Binder & French 1994; French *et al.* 1994), and root buds artificially inoculated with teliospores in the laboratory resulted in systemic infection (French & Lightfield 1990). However, the process of systemic infection in nature remained elusive. It was suggested that systemic infection resulted from incidental contact of root buds with teliospores in the soil (Frantzen 1994). However, research by Friedli & Bacher (2001a) indicates that ovipositional punctures by female *Apion onopordi* results in systemic infection the year following weevil attack. Their research suggests that a mutualistic relationship exists between *A. onopordi* and *P. punctiformis*, whereby the weevil benefits

from increased fitness on infected plants, and the rust benefits by dispersal and increased systemic infection (Friedli & Bacher 2001b; Bacher & Friedli 2002).

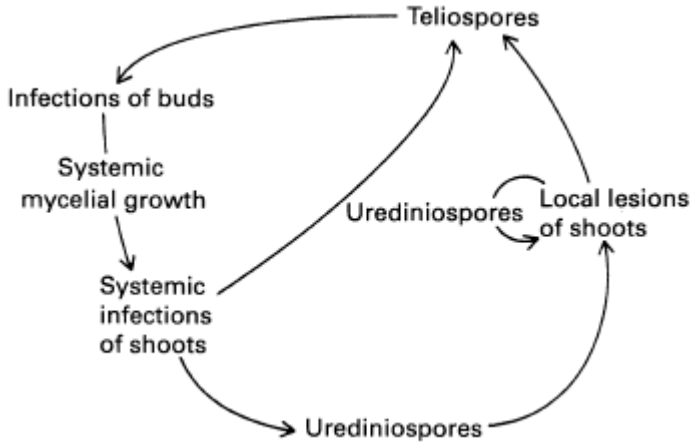

Figure 2.1. Infection cycle of *Puccinia punctiformis* on *Cirsium arvense* (from Frantzen 1994).

Ceratapion onopordi Kirby (Coleoptera: Brentidae) (Fig. 3.3) is a small weevil reported to be oligophagous on the tribe Cardueae (Zwölfer 1965). Recent host specificity testing indicates that development of this weevil is restricted to the subtribes Carduinae and Centaureinae (Gassmann *et al.* 2006). Research indicating that *A. onopordi* is important for promoting systemic infection of *P. punctiformis* on *C. arvense* (Friedli & Bacher 2001a) created interest in this weevil as a biological control agent. The weevil by itself causes minimal impact on *C. arvense*; however, combined with the rust fungus, *P. punctiformis*, the impact on *C. arvense* is believed to be synergistic (Friedli & Bacher 2001b). Furthermore, the fitness of the weevil is increased when developing on rust-infected *C. arvense* (Bacher *et al.* 2002). In the field, *A. onopordi* shows a preference for *C. vulgare*, compared with uninfected *C. arvense* plants (Friedli & Bacher 2001b). In spring, females create ovipositional chambers in the stem base of the plant. Single eggs are laid in each chamber. Larvae mine above and below-ground in the stem, and also pupate in the stem. Adults emerge in autumn and overwinter in leaf litter (Friedli & Bacher 2001b).

Cassida rubiginosa Müller (Coleoptera: Chrysomelidae) (Fig. 3.4) is a leaf feeding beetle belonging to the subfamily Cassidinae, commonly known as tortoise beetles. *C. rubiginosa* is one of a complex of *Cassida* species feeding on thistle host plants. Within the species complex, *C. rubiginosa* is the most widely distributed, occurring across the Palearctic region (Zwölfer & Eichhorn 1966). Zwölfer (1969)

reported *C. rubiginosa* feeding on several plant species in the subtribes Carduinea and Centaureinae, with only slight feeding on *Helianthus* and *Echinops* under starvation conditions. *C. rubiginosa* is reported to occasionally cause serious defoliation of *C. arvense* in North America (Tipping 1993), where it was accidentally introduced sometime before 1901.

Adults overwinter in leaf litter, and emerge in early spring and feed on the first thistle plants available. Eggs are laid in cases (ootheca) usually on the underside of leaves. There are five larval instars, with the first three typically feeding on the leaf underside, and the last two on the upper side (Ward & Pienkowski 1978a). Larvae of *C. rubiginosa* carry a faecal shield, which can protect it against predators (Eisner *et al.* 1967). However, the varying successes of *C. rubiginosa* as a biocontol agent in North America have been attributed to high rates of parasitism (Ward & Pienkowski 1978b; Tipping 1993). In addition, the wasp, *Polistes dominulus* (Hymenoptera: Vespidae), is noted to be a very successful predator of *C. rubiginosa*, in the beetles' native range of Europe (Bacher & Luder 2005). Two adventive *Polistes* species occur on the North Island and northern South Island of New Zealand that may attack introduced *C. rubiginosa* in those regions (Clapperton 1999).

Tipping (1993) noted that there was no difference in the number of *C. rubiginosa* larvae on rust-infected compared with healthy *C. arvense*. A more recent study by Kluth *et al.* (2001) showed that *C. rubiginosa* shows a preference for uninfected, compared with rust-infected *C. arvense*. Furthermore, it has been suggested that the relationship between *C. rubiginosa* and *P. punctiformis* is antagonistic (Kluth *et al.* 2002). Kruess (2002) also indicated that *C. rubiginosa* showed a preference for healthy thistles compared with thistles infected with *Phoma destructiva*.

2.2 PLANT INVASION HYPOTHESES

Invasive plants are often considered to be more vigorous in their introduced ranges (Elton 1958; Crawley 1987); however, since it is estimated that approximately 1 in 1000 introduced plant species become invasive (Williamson 1996) it is not prudent to state any general trends about introduced plants (Simons 2003). Nevertheless, in order to successfully manage the few introduced plants that become invasive weeds, it is important to determine the mechanisms by which they become invasive. A serious criticism of invasive weed research has been that most accounts of invasive weeds being more vigorous in their introduced range are largely anecdotal and observational (Thébaud &

Simberloff 2001). Current research on invasive weeds recognizes the need to move from the mere anecdotal and observational to the quantifiable and predictable (Hinz & Schwarzlaender 2004; Hierro *et al.* 2004).

The most common and familiar theories on the mechanisms of invasion by introduced species are founded in traditional community ecology concepts that predict increased diversity will create increased stability (e.g. Clements 1936; Elton 1958; Pimentel 1961; MacArthur 1970), and thereby resist invasion by exotic species (Elton 1958). This traditional view of community ecology formed the basis of the niche opportunity concept, and the enemy release hypothesis (ERH) (Shea & Chesson 2002).

2.2.1 Niche opportunity concept

The niche opportunity concept suggests that exotic species often fail to invade due to minimal niche opportunities that result from competitive interactions with native species (competitors, pathogens or herbivores) (Elton 1958; Maron & Vilà 2001; Shea & Chesson 2002). It is predicted that "niche opportunities" will decrease with increasing community species richness, due to a more complete use of available resources (Shea & Chesson 2002); however, this prediction is equivocal (Prieur-Richard & Lavorel 2000). Comparative field studies of *Lythrum salicaria* revealed that percent cover was lower in the native region, and that plant species diversity was higher in the native communities (Bastlová-Hanzélyová 2001). This indicates that in the exotic region *L. salicaria* was able to competitively exclude native vegetation, although the precise mechanisms of this invasion are uncertain. Resource availability, as opposed to species richness can also influence invasibility of a community (Foster *et al.* 2002). Fluctuating resource availability has been shown to promote invasion whenever the amount of unutilized resources are increased (Davis *et al.* 2000). Similarly, community perturbation is often associated with release of resources, which in turn creates niche opportunities, and avenues for weed invasion (Drake *et al.* 1989). Disturbance is not always necessary to create a niche conducive for invasion. An invader could have a greater rate of resource acquisition or a lower resource maintenance requirement (Shea & Chesson 2002). An invader could also have a particularly favourable response to a given resource, or environmental condition in the exotic community. The success of *Centaurea solstitialis* invasion has been attributed in part to the deep root system allowing the plant to access water below 60m, which is not used by other plants in the community (Roché 1994)

Soil microbial diversity studies have demonstrated that soil microbes are a determinant of plant community diversity (van der Heijden *et al.* 1998, 1998a), and that invasive plants tend to be more vigorous when grown in exotic soil, likely due to less accumulation of pathogenic microbes (Klironomos 2002). It is also possible that soil microorganisms may affect resources, which in turn can affect plant competitive ability. Callaway *et al.* (2000) studied the effects of soil fungi on the interactions between *Centaurea melitensis*, an exotic weed and *Nassella pulchra*, a native grass species. They discovered that the biomass of *C. melitensis* was significantly lower when soil fungi were reduced, and more importantly they discovered that *C. melitensis* growing with *N. pulchra,* in the presence of soil fungi was able to fully compensate for 30-90% defoliation. This indicates that the exotic weed, *C. melitensis* was able to capitalize on the association with the native grass, *N. pulchra*. Furthermore, the biomass of *N. pulchra* was lowest when grown with defoliated *C. melitensis* in the presence of soil fungi. This result suggests that the biomass of *N. pulchra* was reduced through a strong compensatory growth response in *C. melitensis* mediated by the association with *N. pulchra*. Ridenour & Callaway (2003) also had similar results working with *Centaurea maculosa* in competition with *Festuca idahoensis* in the presence of the biological control agent, *Agapeta zoegana*. These results have an interesting implication, suggesting that a herbivore (or biological control agent) may actually contribute to the competitive ability of an invasive plant (Pearson & Callaway 2003).

2.2.2 The enemy release hypothesis

The enemy release hypothesis (ERH) is essentially a component of the niche opportunity concept. The ERH suggests that release from natural enemies may increase the competitive ability of introduced species relative to native species which experience natural enemy attack, and thereby facilitate community invasions. Although there are many factors that can contribute to species invasions, the ERH is the oldest and most commonly asserted explanation for the increased vigour of invasive weeds. Elton (1958) assumed that release from natural enemies was a major contributing factor to the increased vigour of invasive weeds, and Williams (1954) suggested that release from natural enemies was the primary reason that some weeds become invasive: “the more excessive the growth of an introduced weed, the more likely is the cause to be an absence of natural enemies, and the chances of controlling it biologically are correspondingly higher.” The ERH has long been assumed the primary mechanism explaining the success

of classical biological control of weeds. In fact the assumed maxim of the ERH, based on its intrinsic merit has been the premise of many classical biological control programs (Debach & Rosen 1991). Successful classical biological control lends pertinent evidence in support of the ERH, but is by no means a test of the hypothesis.

The ERH posits that upon introduction to an exotic region, plants experience a decrease in herbivore pressure, facilitating their increase in distribution and abundance. The ERH also postulates that introduced weeds will gain a competitive advantage relative to other plants in the community that are attacked by their own suite of herbivores (competitive release) (Keane & Crawley 2002). A critical assumption of the ERH is that herbivores are important regulators of plant population dynamics. Crawley (1989) reviewed the evidence on the impact of herbivores on plant population dynamics and highlighted the need for manipulative experiments testing the impact of insects on plant population dynamics. Studies investigating the impact of natural enemies on plant population dynamics have yielded mixed results, and the outcome appears to depend on the life history of the plant involved, the herbivores involved and environmental conditions (Louda 1983; Crawley 1989; Louda 1994; Louda & Potvin 1995; Root 1996; Maron & Gardner 2000).

The ERH has been cited in many cases as a plausible explanation for the increased vigour of invasive weeds (Jakobs *et al.* 2004; Woodburn & Sheppard 1996; Weiss & Milton 1984; Lonsdale & Segura 1987; Fowler *et al.* 1996; Prati & Bossdorf 2004; Wolfe 2002; DeWalt *et al.* 2004; Buckley *et al.* 2003; Ebeling *et al.* 2008), however, the evidence for the ERH is still equivocal. Probably the best evidence in support of the ERH to date comes from the study of the invasive weed, *Clidemia hirta*, by DeWalt *et al.* (2004). The study by DeWalt *et al.* (2004) excluded natural enemies (insect herbivores and pathogens) in both the native and exotic ranges, and determined that under some habitat conditions, natural enemies were important in suppressing the weed. Recent reviews have emphasized the need for biogeographic studies in order to elucidate mechanisms of plant invasions and to assess the impacts of natural enemies on weeds in order to improve classical biological control (Hinz & Schwarzlaender 2004; Hierro *et al.* 2004).

Figure 2.2. *Cirsium arvense* shoot infected with the rust pathogen, *Puccinia punctiformis*. Pukeatua, New Zealand, January 2007.

Figure 2.3. Adult of the stem-mining weevil, *Ceratapion onopordi*, on *Cirsium vulgare*. St. Ursanne, Switzerland, May 2008.

Figure 2.4. Adult (top) and larva (bottom) of the leaf-feeding beetle, *Cassida rubiginosa*.

Chapter 3

Enemy release does not increase performance of *Cirsium arvense* in New Zealand

3.1 INTRODUCTION

Invasive plants can have severe detrimental ecological (Vitousek *et al.* 1997; Mooney & Hobbs 2000) and economic (Pimentel *et al.* 2005) impacts. A common assumption is that these detrimental effects are incurred primarily in the introduced ranges, since invasive plants are often considered more vigorous in their exotic, compared to their native range. There has been a long history of anecdotal reports of introduced plants being more vigorous in their exotic range, but relatively little quantification of these observed differences (Thébaud & Simberloff 2001). For instance, in Canterbury New Zealand early reports of invasive watercress were stated to "attain a size and strength quite unknown in its native country", but no measure of size or vigour was provided (Armstrong 1879). Crawley (1987) also noted observational reports of alien plants growing larger in their introduced range, and presented a comparison of plant heights from floral records that supported this claim. However, mere observational notes and data from floral records that typically report only the size range of some plant parts are insufficient to demonstrate that an introduced plant performs better in its exotic range. Conducting comparative biogeographical studies of invasive plants in their native and introduced ranges has been recognized as an important step towards understanding plant invasions (Hierro *et al.* 2005). A review of quantitative studies comparing plants in their native vs. introduced range showed that the majority of invasive plants are more vigorous in their exotic range, although this was not always consistent (Hinz & Schwarzlaender 2004).

The mechanism most commonly invoked to explain the increased performance of invasive weeds is release from natural enemies (Maron & Vilà 2001; Keane & Crawley 2002; Mitchell & Power 2003). The enemy-release hypothesis (ERH) asserts that upon introduction to an exotic range, plants experience a decrease in natural enemy pressure that facilitates their dispersal and increased abundance. Introduced plants can experience direct fitness benefits from decreased natural enemy pressure, and may also experience selection for more vigorous genotypes that invest more in growth and reproduction, and less in defence against herbivores and pathogens (Blossey & Nötzold 1995). Studies

comparing herbivory and the natural enemy complexes in native and introduced ranges have found reduced herbivory, lower overall diversity of natural enemies, and a shift from specialists to generalists in the introduced range of invasive plants (e.g. Goeden 1974; Memmott *et al.* 2000; Wolfe 2002; Hinz & Schwarzlaender 2004; Colautti *et al.* 2004; Genton *et al.* 2005; Cripps *et al.* 2006; Liu & Stiling 2006).

Successful classical biological control also lends pertinent evidence in support of the ERH, but does not dictate that plants are more vigorous in their introduced range due to lack of natural enemies. Although the implicit principle of the ERH has been the premise of most classical biological control programs, in most cases little is known about how an invasive plant grows in its native range (Hierro *et al.* 2005). In New Zealand (NZ) there is renewed interest in biological control of *Cirsium arvense* (L.) Scop. As part of the ongoing biological control effort against this weed in NZ, field surveys were carried out in Europe and the North and South Islands of NZ in order to quantitatively compare the performance of the plant and the levels of herbivory among ranges. Our purpose for conducting these surveys was two-fold: Firstly, to provide baseline data on the growth of *C. arvense* in NZ that could be used for subsequent assessment of the impacts of biological control agents; and secondly to test assumptions of the ERH, which has implications for classical biological control. In line with the assumptions of the ERH, we hypothesized that (1) plant performance would be greater in the introduced ranges of the North and South Islands of New Zealand, and (2) herbivory would be greater in the native range.

3.2 METHODS

3.2.1 Study system

Cirsium arvense (L.) Scop. (Asteraceae) (Californian, Canada, or creeping thistle) is a perennial herb and a member of the tribe Cardueae, which comprises the plants commonly known as thistles (Bremer 1994). It is indigenous to Eurasia but has been accidently spread throughout temperate regions of the world, where it is considered one of the worst invasive weeds (Holm *et al.* 1977). It was first reported as an accidental introduction to NZ in 1878 (Kirk 1878), and is presently considered one of the worst weeds in NZ arable and pastoral production systems (Bourdôt & Kelly 1986; Bourdôt *et al.* 2007). *Cirsium arvense* spreads clonally by means of creeping lateral roots, and also reproduces by seeds. *Cirsium arvense* is almost completely dioecious with individual plants having capitula containing either male (staminate) or female (pistillate) florets. Seed production is

dependent on insect pollination and proximity to male plants. Population sex ratios range from equal male:female ratios (Lloyd & Myall 1976) to extremely female biased sex ratios (Lalonde & Roitberg 1994). In general, few seeds are produced where male and female plants are separated by more than 50m (Lalonde & Roitberg 1994). In New Zealand, *C. arvense* flowers from December to February, and seeds are produced from December to April (Webb *et al.* 1988). In the native range of Europe, *C. arvense* flowers from July to September (Clapham *et al.* 1987), and seeds are also produced from July to September (M. Cripps, personal observation).

There is a long history of control efforts against *C. arvense*, including cultural, chemical and biological methods (Donald 1990). Classical biological control has been attempted in Canada, USA and NZ. In North America biological control of thistles in general was hampered by evidence that *Rhinocyllus conicus* (Frölich) was negatively impacting populations of related native thistles (Louda *et al.* 1997; Louda 1999). However, in NZ there are no native plants in the tribe Cardueae (Webb *et al.* 1988), enabling the recent release of two oligophagous thistle herbivores from Europe: *Cassida rubiginosa* Müller and *Ceratapion onopordi* Kirby. Previously, from 1979 to 1996, four insect herbivores [*Altica carduorum* Guér., *Hadroplontus litura* (F.), *Lema cyanella* (L.), and *Urophora cardui* (L.)] had been released in NZ for biological control of *C. arvense* (Julien & Griffiths 1998), but all have failed to establish (Harman *et al.* 1996), except for *L. cyanella*, which has reportedly established at one site in the North Island (Landcare Research, unpublished data). Additionally, *R. conicus* was released for control of *Carduus nutans* L. in 1973 (Julien & Griffiths 1998), and is known to also attack *C. arvense* (Zwölfer & Harris 1984). Other than insect herbivores, the highly specialized rust fungus, *Puccinia punctiformis* (Str.) Röhl., is also known to occur on *C. arvense* in NZ, and was present as early as 1881 (Cunningham 1927).

3.2.2 Field surveys

In summer of 2007, a survey of *C. arvense* was conducted in 13 populations in NZ, (7 in the North Island, and 6 in the South Island) and in 12 populations in Europe (Table 3.1). The survey in NZ was carried out in the lower latitude region of the North Island and the higher latitude region of the South Island (Table 3.1), where *C. arvense* is considered a serious problem. The survey in central Europe was carried out in two areas of similar latitude. The first survey area was in Germany, France and Switzerland, and the second in Hungary and Croatia (Table 3.1). For practical reasons, no attempt was made to randomly

scatter sites over the surveyed ranges, so extrapolation of results to the whole of NZ and Europe is not possible. The intention was simply to find a good number of populations subject to natural enemy attack in Europe for comparison with populations not subject to such attack in the North and South Islands of NZ. All surveys were carried out on agricultural land during the flowering to fruiting period of the plant when shoots had achieved maximum growth. In NZ this was from late January to mid February, and in Europe from June to early August. In both ranges only relatively large populations (at least 20m diameter) were selected in order to achieve replication within a population. At each field site the land area occupied by the *C. arvense* population was estimated. A population was defined in this study as a continuous patch without separation between adjacent shoots of more than 50m, since little exchange of pollen occurs between patches separated by more than this distance (Lalonde & Roitberg 1994). A transect of up to 40m was randomly placed within each population. Quadrats ($1m^2$) were systematically placed at 2m intervals along the transect up to a maximum of 20 quadrats. In each quadrat the number of *C. arvense* shoots was counted to provide a measure of shoot density. Visual percent cover estimates were also made for the proportion of *C. arvense*, grasses, herbs, and bare ground per quadrat that totalled to 100 percent. Additionally, at every second quadrat along each transect (maximum of 10 quadrats) aerial shoots were harvested and brought back to the laboratory. In the laboratory the height (cm) of each aerial shoot was measured, and the stems and capitula of 3 shoots randomly selected from each quadrat (maximum of 30 shoots per population) were dissected to examine for endophagous herbivory. All the shoots from each harvested quadrat were then placed in a drying oven at 70°C for approximately 48 hrs to measure above-ground dry weight (g) per quadrat.

Climatic data was gathered from both ranges in order to assess weather conditions that could account for differences in plant growth. For the European range, weather data were collected from the National Climate Center database (http://mi3.ncdc.noaa.gov/), and for NZ, weather data were collected from the National Institute of Water and Atmospheric Research (http://cliflo.niwa.co.nz/). For each surveyed population of *C. arvense* the data from the closest available weather station were used. Distance between the surveyed population and the nearest weather station ranged from 3 to 54 km. For each population daily means were collected for each climatic metric, and 10-year means were calculated for annual mean temperature, mean temperature of the warmest month, mean temperature of the coldest month, and total annual precipitation.

Table 3.1. Populations of *Cirsium arvense* surveyed in Europe and New Zealand North and South Islands with corresponding 10-year mean climatic data.

Population code	Country/ Region	Coordinates*		Altitude (m)	Mean annual temperature (°C)	Mean max temperature of warmest month (°C)	Mean min temperature of coldest month (°C)	Mean annual precipitation (mm)
Europe								
CH1	Switzerland	N 46° 58.906'	E 007° 08.443'	432	10.6	25.7	-1.4	986
D1	Germany	N 47° 52.784'	E 007° 35.247'	204	10.9	27.4	-2.7	551
D2	Germany	N 47° 48.898'	E 007° 35.377'	225	10.8	27.2	-2.7	783
F1	France	N 47° 26.398'	E 007° 18.103'	573	10.3	25.5	-2.3	755
H1	Hungary	N 47° 42.428'	E 016° 34.532'	235	10.8	27.9	-4.4	465
H2	Hungary	N 47° 39.996'	E 016° 40.085'	135	10.8	27.9	-4.4	465
H3	Hungary	N 47° 39.349'	E 016° 51.744'	109	10.8	27.9	-4.4	465
H4	Hungary	N 47° 33.980'	E 017° 04.796'	126	10.8	27.9	-4.4	465
H5	Hungary	N 47° 15.953'	E 017° 09.669'	138	11.0	28.4	-5.1	546
H6	Hungary	N 46° 49.126'	E 016° 34.739'	268	10.5	28.3	-6.7	753
HR1	Croatia	N 46° 12.608'	E 016° 44.040'	150	11.2	28.0	-5.3	752
HR2	Croatia	N 45° 31.974'	E 018° 01.300'	112	11.4	28.2	-4.8	661
NZ North Island								
BP1	Bay of Plenty	S 37° 26.952'	E 175° 51.557'	124	14.8	25.7	4.7	1224
BP2	Bay of Plenty	S 37° 33.365'	E 175° 54.397'	10	15.1	24.7	5.6	1225
BP3	Bay of Plenty			10	15.1	24.7	5.6	1225
W1	Waikato	S 38° 03.640'	E 175° 32.667'	252	14.0	25.8	3.0	961
W2	Waikato			252	14.0	25.8	3.0	961
AK1	S. Auckland	S 37° 35.065'	E 175° 06.852'	28	13.9	25.1	3.1	1200
AK2	S. Auckland			28	13.9	25.1	3.1	1200
NZ South Island								
O1	S. Otago	S 46° 09.438'	E 169° 32.975'	104	10.5	21.2	1.0	662
SL1	Southland	S 46° 06.972'	E 168° 01.748'	82	10.4	21.3	0.9	891
SL2	Southland	S 46° 09.395'	E 167° 36.358'	157	10.1	19.7	0.9	1127
SL3	Southland			157	10.1	19.7	0.9	1127
SL4	Southland	S 46° 11.538'	E168°02.422'	70	10.4	21.3	0.9	891
SL5	Southland			70	10.4	21.3	0.9	891

* Populations without coordinates were within 1 km of the preceding population.

3.2.3 Data analyses

Data analyses occurred in three stages. Firstly, data from individual quadrats were used to test for differences among populations within each range for shoot density, shoot dry weight, shoot height, and percent cover estimates of *C. arvense*, other herbaceous plants, grasses and bare ground, using one-way analysis of variance. Secondly, sample means for each population were used to test for differences among ranges (Europe, NZ North Island, and NZ South Island) in each of these variables, again using one-way analysis of variance. The proportion of shoots attacked among ranges was analysed by using a generalized linear model (GLM) with a logit-link function, allowing for over-dispersion and assuming a binomial distribution (with the binomial total being the sum of the total number of dissected shoots). Analyses for shoot attack were done separately for capitula and stem attack, respectively. Thirdly, analysis of covariance incorporating the 10-year mean annual precipitation and altitude for each population was used to adjust for abiotic differences which could potentially explain differences in growth patterns among ranges. Mean temperatures for each population are reported in Table 3.1 but not included in the analysis since lack of overlap among the ranges would confound the analysis. All analyses were conducted using GenStat (Version 11).

3.3 RESULTS

Variation among populations within each of the three ranges was significant ($P<0.001$) for all plant traits measured (Fig. 3.1). There was a great degree of variation in the land area occupied by the populations surveyed in all ranges, with individual populations ranging in size from 82 to 15,000m^2 in Europe, from 180 to 3,000m^2 in the North Island and from 800 to 9,000m^2 in the South Island. The difference in population sizes among the ranges was not significant ($P=0.412$). Shoot height was significantly smaller in the North Island compared to the South Island, but shoot height in Europe was not significantly different from either NZ range (Fig. 3.2A, Means). Shoot dry weight was significantly greater in the South Island compared to the North Island and Europe (Fig 2.2B, Means). Shoot density was not significantly different among the three ranges (Fig. 3.2C, Means). When comparing visual estimates of percent plant cover there was no difference between ranges in the cover of *C. arvense*, other herbaceous plants, and grasses; but there was significantly more bare ground in Europe compared to both the North and South Islands of NZ (Fig. 3.3, Means).

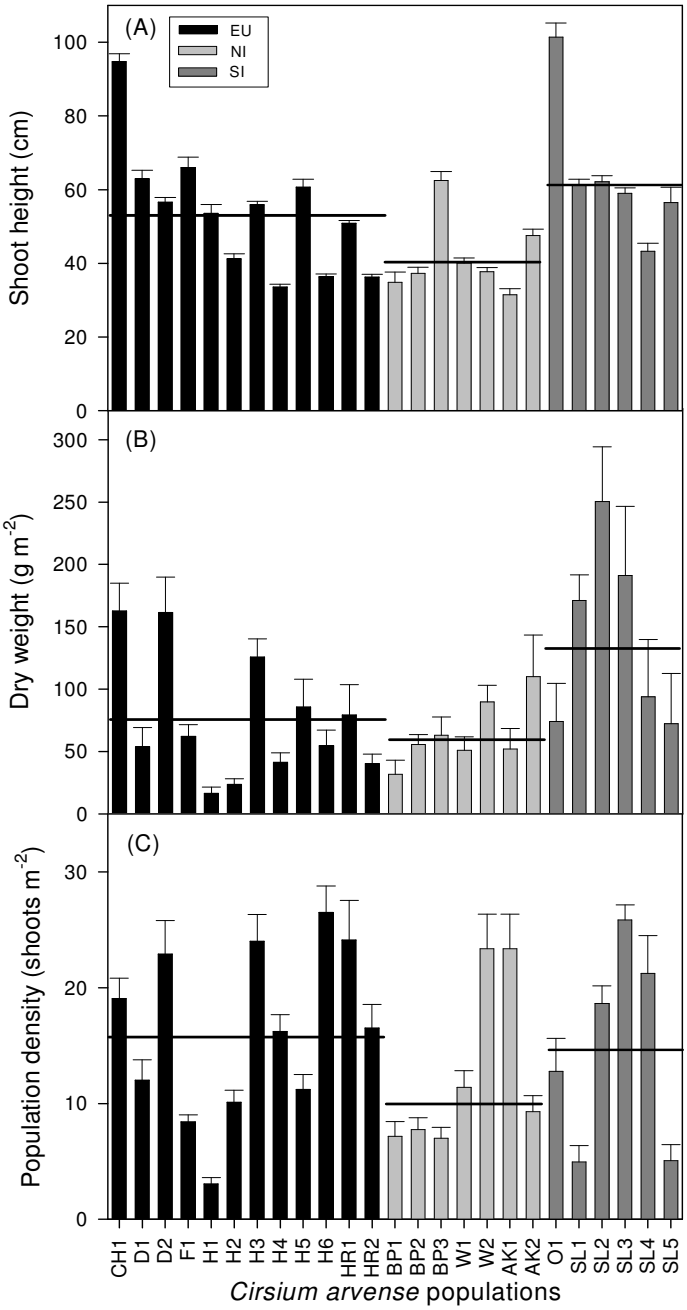

Figure 3.1. Mean (±SE) shoot height (A), dry weight (B), and population density (C) for *Cirsium arvense* populations surveyed in Europe (EU), NZ North Island (NI) and NZ South Island (SI), 2007. The horizontal lines through the bars indicate the mean for each range.

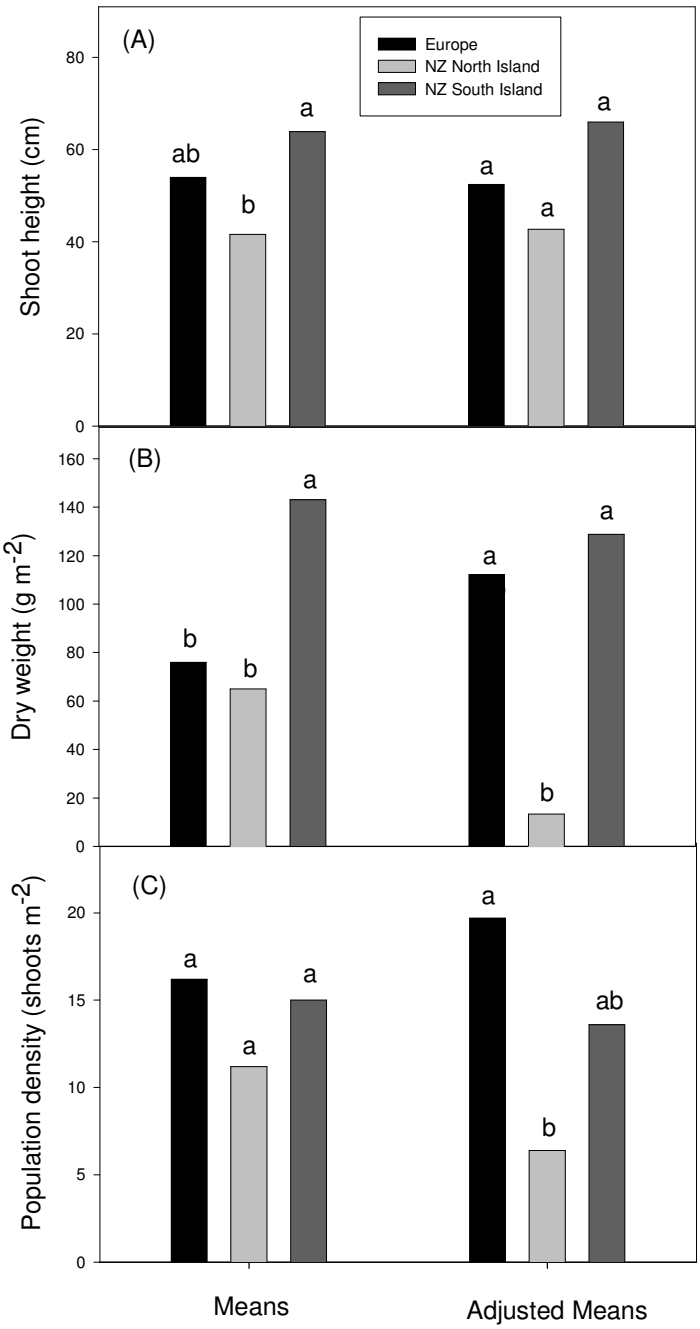

Figure 3.2. Mean shoot height (A), dry weight (B) and population density (C) for *Cirsium arvense* populations surveyed in Europe, NZ North Island and NZ South Island, 2007. Means and adjusted means (using altitude and precipitation as covariates) are compared using unrestricted least significant difference (LSD 5%). Bars within a category (Means or Adjusted Means) that have the same letter are not significantly different. LSD values for Europe compared with the South Island for height, dry weight and density respectively are: 16.74, 53.40, and 7.55; and when adjusted for covariates LSD values are: 26.23, 72.90, and 11.85.

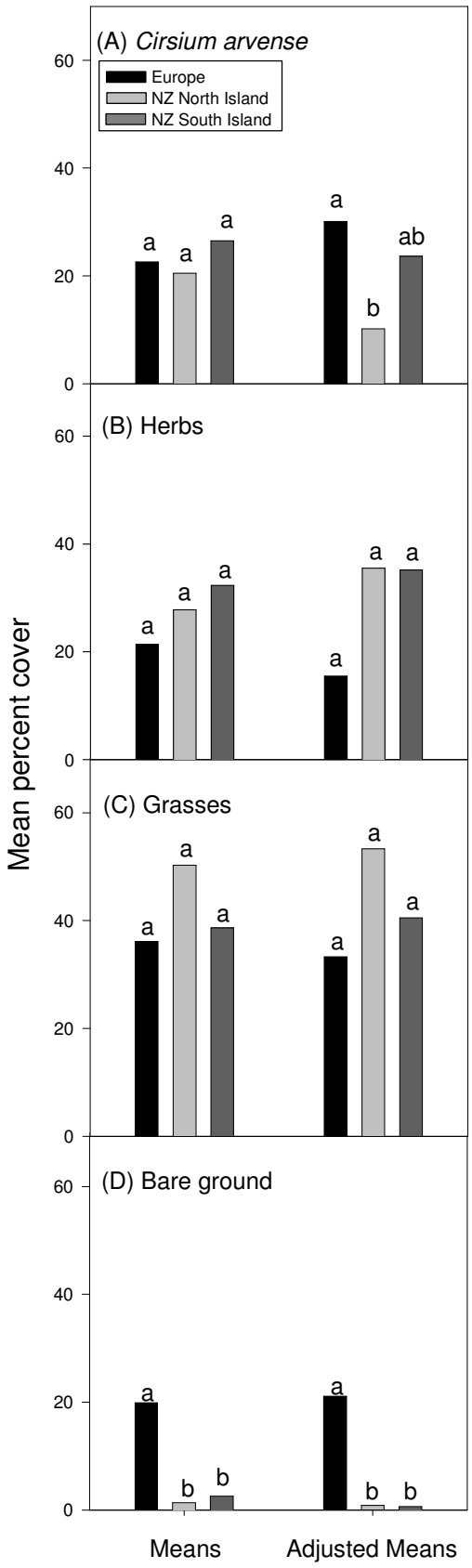

Figure 3.3. Mean percent cover estimates of *Cirsium arvense*, herbaceous plants, grasses, and bare ground occurring in sampled quadrats ($1m^2$) in populations of *C. arvense* surveyed in Europe, NZ North Island, and NZ South Island, 2007. Means and adjusted means (using altitude and precipitation as covariates) are compared using unrestricted least significant difference (LSD 5%). Bars within a category (Means or Adjusted Means) that have the same letter are not significantly different. LSD values for Europe compared with the South Island for *C. arvense*, grasses, herbaceous plants and bare ground respectively are: 12.3, 17.1, 21.3, and 10.7; and when adjusted for covariates LSD values are:18.2, 27.9, 35.2, and 14.5.

Capitula attack by endophagous herbivores was significantly greater in Europe (mean % capitula attacked ± SE = 49.6%±6.4) compared to the NZ North Island (mean % capitula attacked ± SE = 23.8%±6.7) (deviance ratio=20.5; $P< 0.001$; Fig. 3.4A, B). No capitula attack was found in the NZ South Island (Fig. 3.4B). The percentage of stem attack was also significantly greater in Europe, with an overall mean (±SE) of 14.4%±4.8; no stem attack was found in either the North or South Islands of NZ (deviance ratio=23.8; $P<0.001$; Fig. 3.4C, D). All endophagous herbivory in NZ was from the capitulum-feeding weevil, *Rhinocyllus conicus*, whereas in Europe a great variety of insect herbivores attacked inside the capitula and stems of the plant.

Latitude and all climatic variables included were significantly different among all three ranges ($P<0.001$; Table 3.1). Precipitation was greatest in the NZ North Island followed by the NZ South Island, and lowest in Europe. Altitude was significantly greater in Europe compared to the North Island, but not different from the South Island; the North and South Island were not significantly different in altitude.

When adjusted for covariates (altitude and precipitation) Europe and the South Island were not different for all three plant traits measured (Fig. 3.2, Adjusted Means). After adjustment for covariates shoot height was not significantly different among the three ranges (Fig. 3.2A, Adjusted Means); dry weight was significantly less in the North Island compared to the South Island and Europe (Fig. 3.2B, Adjusted Means), and shoot density was significantly less in the North Island compared to Europe (Fig. 3.2C, Adjusted Means). The adjustments for altitude and precipitation are depicted for dry weights in Figure 3.5. Adjusting to a common precipitation had a significant ($P=0.008$) effect that caused dry weight to increase for Europe, and decrease for both the NZ ranges (Fig. 3.5A); but adjusting to a common altitude had no significant effect on dry weight among the ranges ($P=0.252$; Fig. 3.5B). After adjustment for covariates the percent cover of *C. arvense* was lower in the North Island compared to Europe, but other herbaceous plants, and grasses remained non-significantly different among ranges, and the difference in bare ground remained significantly greater in Europe compared to either NZ range (Fig. 3.3, Adjusted Means).

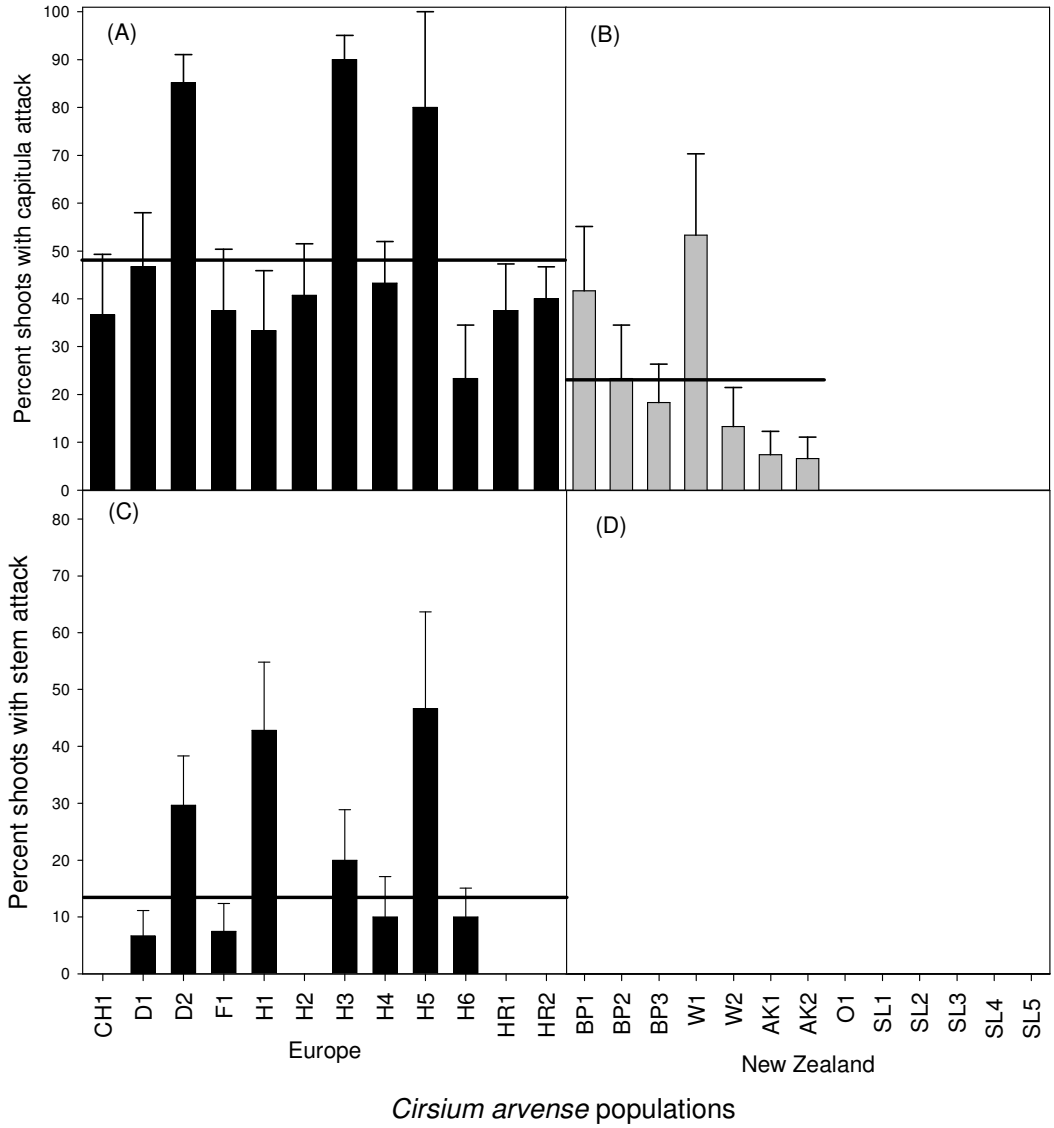

Figure 3.4. Mean (±SE) percent of shoots with endophagous herbivore attack for surveyed populations of *Cirsium arvense* in Europe (black) and the North and South Islands of NZ. (A) Percent shoots with capitula attack in Europe. (B) Percent shoots with capitula attack in NZ. Capitula attack was only found in the NZ North Island (grey). (C) Percent shoots with stem attack in Europe. (D) No stem attack was found in either NZ range. Statistical inferences were based on logit transformed data. The horizontal lines through the bars indicate the mean for each range.

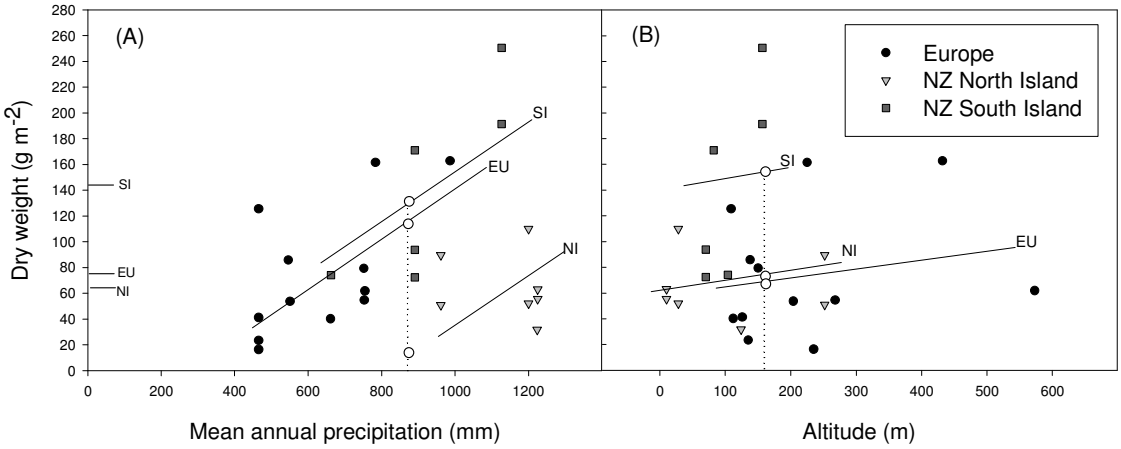

Figure 3.5. Mean dry weight showing the adjustments for precipitation (A) and altitude (B) for each surveyed range (EU=Europe, NI=North Island, SI=South Island). The unadjusted means (after ANOVA) for each range are shown along the y-axis. The solid lines are regression lines for each range adjusted to a common slope. The dotted vertical lines indicate the mean precipitation (849mm) and altitude (162m) for all three ranges, and the open circles at the intersection of the regression lines and the common covariate values show the adjusted means after ANCOVA.

3.4 DISCUSSION

Contrary to our first hypothesis we failed to find any convincing evidence that *C. arvense* is more vigorous in its introduced range. Dry weight was greater in the South Island, which is in line with our prediction of increased performance in the introduced range; however, this was explained by more favourable conditions (i.e. increased precipitation in NZ) and therefore does not support the hypothesis that increased vigour is due to release from natural enemies. In the introduced range of the NZ North Island plant performance was not different than Europe for any plant trait compared and after adjusting for differences in altitude and precipitation shoot density and dry weight were in fact lower in the North Island, which is contrary to the ERH and our first prediction. This would suggest that the similar performance of *C. arvense* in the North Island and Europe is due to more favourable environmental conditions for *C. arvense* growth in the NZ North Island.

The general lack of increased performance of *C. arvense* in both introduced NZ ranges is in contrast to most other similar studies, which indicate that invasive weeds are more vigorous in their introduced range (Hinz & Schwarzlaender 2004). However, only a limited number of such comparative surveys have been conducted, and it has been noted that many of these conducted to date have been rather low in quality (e.g. low number of

populations surveyed, few plant traits measured, herbivory not reported, no weather data) (Hinz & Schwarzlaender 2004). Recently, more rigorous comparative surveys have produced evidence that the invasive weeds *Solidago gigantea* and *Buddleja davidii* grow more vigorously in their introduced range, and that this increased growth performance cannot be attributed to more favourable climatic conditions, which lends support for the ERH (Jakobs *et al.* 2004; Ebeling *et al.* 2008). In contrast, our data indicate that the increased dry weight of *C. arvense* in the South Island can be explained by more favourable abiotic conditions and even the similar performance of *C. arvense* in the North Island compared to Europe can be attributed to more favourable conditions without which the plant's growth would be reduced to levels significantly less than in its native range.

The general decreased performance of *C. arvense* in the North Island might be due to other environmental factors associated with lower latitudes. For instance temperature was significantly different in the North Island compared to the other ranges, but could not be incorporated into the covariate analysis due to lack of overlap among the ranges, which would have confounded the analysis. Thus, if other covariates could be used the results might be different, which indicates that the data presented here should be interpreted with some caution. The idea that other environmental factors might contribute to reduced growth of *C. arvense* in the North Island is supported by the fact that *C. arvense* is not a problematic plant at latitudes lower than 37° North or South in North America (Moore 1975) and Australia (Armor & Harris 1974), respectively. Thus, our data also indicate that the plant is a more vigorous weed in higher latitude temperate conditions, and additionally show that this is regardless of whether it is in the introduced or native range.

The lack of evidence for increased vigour of an invasive plant in its introduced range is not completely unique. Whether or not a plant is considered more or less vigorous in its introduced range often depends on the trait(s) measured. For instance, *Mimosa pigra*, *Heliotropium europaeum* and *Rhododendron ponticum* all showed a general increased vigour in the introduced range, but for some traits equal or reduced growth in the introduced range (Lonsdale & Segura 1987; Sheppard *et al.* 1996; Erfmeier & Bruelheide 2004). In other cases reduced plant performance in the introduced range was considered to be related to nutrient conditions of the particular habitat (Edwards *et al.* 1998). In the case of broom, *Cytisus scoparius*, Paynter *et al.* (2003) found no difference in plant size and growth rate between its native and introduced ranges, but a significantly greater population density in the introduced range. It was suggested that the similar size and growth rate between ranges might be a result of increased intraspecific competition

due to the higher population densities in the introduced range, and that this may have concealed effects of release from natural enemies that might have been evident at lower densities. In our study, density of *C. arvense* was not different among ranges, and therefore there was no reason to believe that intraspecific competition was significantly different. Additionally there were no differences in the percentage cover of other herbaceous plants and grasses, which might suggest that interspecific competition was also not different. Therefore it is unlikely that competition may have concealed effects of release from natural enemies on individual shoot growth. However, there was significantly more bare ground in the European range, which would suggest greater disturbance that could allow for increased growth and spread of the plant. Interspecific competition from grasses is known to have a strong adverse effect on the growth of *C. arvense*, and microsite availability, such as bare ground, has been shown to be critical for new shoot recruitment (Edwards *et al.* 2000). Nevertheless, when percent cover of other herbs, grasses and bare ground were used as covariates there was no change in the density, height or dry weight of *C. arvense* among ranges.

In accordance with our second hypothesis herbivory was significantly greater in the native range. The phytophagous insect community on *C. arvense*, and high proportions of shoot attack, are well known from its native range in Europe (Zwölfer 1965; Schröder 1980; Freese 1994). Assessing the degree of specialized attack in NZ was considered important since biological control agents have been released for this weed in NZ in the past (Julien & Griffiths 1998), and the possibility of host shifts by indigenous herbivores onto the introduced plant cannot be discounted (e.g. Olckers & Hulley 1991; Creed & Sheldon 1995; Cripps *et al.* 2006). The surveys conducted here confirm the lack of agent establishment on *C. arvense* noted by other authors (Harman *et al.* 1996). The absence of host shifts by indigenous insect herbivores is not surprising since no native plants occur in the Cardueae tribe in NZ. Previously, the degree of attack by *R. conicus* on *C. arvense* in NZ was unknown. Here we show that *R. conicus*, originally released for control of *Carduus nutans*, is also commonly encountered on *C. arvense*, at least in the North Island of NZ. Interestingly, *R. conicus* was not encountered in any of the surveyed populations in the South Island of NZ, although adult weevils have been observed in that region (M. Cripps, personal observation). Similarly, Fenner & Lee (2001) found no endophagous herbivore attack in the capitula of *C. arvense* from a survey carried out in 1998 in the South Island of NZ. The rust pathogen, *P. punctiformis*, is known to cause severe detrimental effects, often killing shoots before flowering (Watson & Keogh 1980;

Thomas *et al.* 1994), and the proportion of shoots attacked by this pathogen has been shown to be similar in both NZ and Europe (Chapter 4; Cripps *et al.* 2009).

The fact that endophagous herbivory was much greater in the native range, but that plant performance was generally not different, or explained by environmental differences among ranges could indicate that *C. arvense* is not influenced by endophagous herbivory. Another possibility is that the relatively low natural enemy pressure we have found in NZ is sufficient to maintain plant growth at similar levels to the native range. Successful biological control is often attributed to a single best agent (Denoth *et al.* 2002). Thus, it is possible that the specialized natural enemies present in NZ (i.e. *R. conicus* and *P. punctiformis*) are sufficient to decrease the plant performance to levels similar to the native range. However, this is unlikely, since capitulum feeders have been shown to have negligible influence on the population dynamics of *C. arvense* (Edwards *et al.* 2000), which is further supported by data showing that seedlings do not contribute to the growth of established *C. arvense* populations (Bourdôt *et al.* 2006). And, although *P. punctiformis* can kill individual shoots, population effects are minimal, even with artificial manipulation (Frantzen 1994; Kluth *et al.* 2003). In terms of generalist insect herbivores, only five have been reported in association with *C. arvense* in NZ (Spiller & Wise 1982), and the only insects commonly encountered on *C. arvense* in NZ were pollinators (M. Cripps, personal observation). Therefore it is also unlikely that generalist insect herbivores have an impact on *C. arvense* in NZ.

In conclusion, addressing the hypothesis of increased plant performance in the introduced range compared to the native range is important in order to gain a more objective perspective on invasive weeds, and to better inform management decisions, particularly biological control. In accordance with the ERH, it is evident that *C. arvense* is released from natural enemy pressure in NZ. However, this release from natural enemies has not resulted in increased performance of *C. arvense*, and does not explain the case of increased dry weight in the South Island. As a result, the data presented here do not offer promising support for the classical biological control of *C. arvense*; however, no strong conclusion can be made, since any introduced agent would in turn be released from its own specialized predators and parasitoids, which could allow it to have an unexpected impact on the plant. This study was the first step in our biogeographic comparison of *C. arvense* in its native and introduced ranges. Further experimental study was also carried out to assess population demographics in the native and introduced range when herbivores and pathogens were excluded, or not. This will be reported on subsequently (Chapter 5)

and will offer more insights into the influence of natural enemies on the population dynamics of *C. arvense.*

Chapter 4

Does transmission of the rust pathogen, *Puccinia punctiformis*, require stem mining vectors?

4.1 INTRODUCTION

Cirsium arvense (L.) Scop. (Californian, Canada, or creeping thistle) is one of the worst weeds in New Zealand (NZ) arable and pastoral production systems (Bourdôt & Kelly 1986; Bourdôt *et al.* 2007). It is a species of Eurasian origin that was accidentally introduced to NZ approximately 130 years ago. Not long after its establishment, the highly specialised rust fungus, *Puccinia punctiformis* (Str.) Röhl., was also noted to be widespread on *C. arvense* (Cunningham 1927). Its potential as a biocontrol agent in NZ did not go unnoticed, but was hampered by an incomplete understanding of the factors affecting the infection process and disease development (Cockayne 1914; 1915). This pathogen is an attractive biocontrol agent because the systemic disease it causes has severe detrimental effects on the plant, often killing shoots before they flower (Watson & Keogh 1980; Thomas *et al.* 1994). However, the infection process remains obscure, in particular, how systemic infection is initiated and how subsequent disease arises. The current understanding is that systemic disease arises from adventitious shoot buds contacting basidiospores from germinated teliospores in the soil (Van den Ende *et al.* 1987; French & Lightfield 1990; Frantzen 1994). However, studies attempting to increase systemic disease by augmenting spore levels have met with limited success. Kluth *et al.* (2003) found that by actively spreading urediniospores (that produce urediniosori that morph into teliosori with teliospore production) onto plants, the number of systemically diseased shoots could be increased the following season; but this did not result in a significant reduction in the number of flowering shoots. Furthermore, experiments applying spore suspensions to the soil did not result in an increase of systemically diseased shoots in field trials (Frantzen & Scheepens 1993).

More recently, there has been increasing evidence suggesting that stem mining insects act as vectors of the pathogen and are largely responsible for systemic infection and disease (Friedli & Bacher 2001a; Wandeler & Bacher 2006; Wandeler *et al.* 2008). This recent research has focused on the oligophagous stem mining weevil, *Ceratapion* (=*Apion*) *onopordi* Kirby. The proposed mechanism is that the female weevil carries urediniospores and inoculates shoots via ovipositional punctures in the stem base (Friedli

& Bacher 2001a; Wandeler & Bacher 2006). This was successfully demonstrated in a natural field population where systemic disease was increased in the vicinity of shoots experimentally infested with spore-covered weevils (Wandeler *et al.* 2008). Several specialised stem mining insects exist on *C. arvense* in the plant's native range (Freese 1994; Zwölfer 1965) that could be capable of vectoring the pathogen in the manner proposed by Friedli & Bacher (2001a). The research indicating that *C. onopordi* is an important vector of the rust pathogen was used to support its approval for field release in NZ. It was assumed that the weevil would work synergistically with the rust pathogen to enhance biological control of *C. arvense* (Friedli & Bacher 2001a; b). Previously the level of rust and the amount of systemic vs. localised disease in NZ was unknown. Here we present comparative survey data on levels of rust in the native (Europe) and introduced (NZ) ranges of the thistle, and question the ecological importance of stem miners for vectoring *P. punctiformis*.

4.2 METHODS

In summer of 2007, surveys of *Cirsium arvense* were conducted in 13 populations in New Zealand, and 12 populations in Europe (Table 4.1). Surveys were carried out in both the North and South Islands of NZ; and in Germany, France, Switzerland, Hungary and Croatia, in Europe (Table 4.1). The surveys were carried out during the flowering to fruiting period of the plant. In New Zealand this was from late January to mid February, and in Europe from June to early August. At each field site the land area occupied by the *C. arvense* population was estimated. A population was defined as a continuous patch without separation between adjacent shoots of more than 50m (Lalonde & Roitberg, 1994). A transect of up to 40m was randomly placed within each population (patch). Quadrats ($1m^2$) were systematically placed at 2m intervals along the transect up to a maximum of 20 quadrats. In each quadrat the number of healthy and rusted *C. arvense* shoots was counted and totalled for each population. Rusted shoots were classified as having either systemic or localised disease. In addition, the shoots were harvested from every second quadrat (maximum 10 quadrats) and the stems of three healthy shoots were dissected (maximum 30 shoots) and examined for stem mining insects that could potentially vector the rust pathogen.

The proportion of diseased shoots was compared between Europe and New Zealand using a generalized linear model (GLM) with a logit-link function, allowing for over-dispersion and assuming a binomial distribution (with the binomial total being the

sum of the total number of shoots in each population). This analysis was also carried out including only those populations where rust was detected. The proportion of quadrats infected out of the total along the transects in the populations that had rust present was analysed in the same way. The three analyses correspond to examining overall disease levels, and incidence of disease where it was present, at the levels of the population and quadrat. Analyses were conducted using GenStat (Version 10.1).

Table 4.1. *Cirsium arvense* populations surveyed in Europe and New Zealand, 2007.

European populations					
Population code	Site Name	Country	Coordinates		Patch size (m^2)
CH1	Müntschemier	Switzerland	N 46° 58.906’	E 007° 08.443’	82
D1	Grissheim	Germany	N 47° 52.784’	E 007° 35.247’	420
D2	Müllheim	Germany	N 47° 48.898’	E 007° 35.377’	1,600
F1	Ligsdorf	France	N 47° 26.398’	E 007° 18.103’	1,500
H1	Sopron	Hungary	N 47° 42.428’	E 016° 34.532’	1,500
H2	Balf	Hungary	N 47° 39.996’	E 016° 40.085’	300
H3	Sarród	Hungary	N 47° 39.349’	E 016° 51.744’	15,000
H4	Babót	Hungary	N 47° 33.980’	E 017° 04.796’	5,000
H5	Celldömölk	Hungary	N 47° 15.953’	E 017° 09.669’	180
H6	Rédics	Hungary	N 46° 49.126’	E 016° 34.739’	7,500
HR1	Legrad	Croatia	N 46° 12.608’	E 016° 44.040’	2,000
HR2	Đurdenovac	Croatia	N 45° 31.974’	E 018° 01.300’	600

New Zealand populations					
Population code	Site Name	Region	Coordinates*		Patch size (m^2)
BP1	Waihi 1	Bay of Plenty	S 37° 26.952’	E 175° 51.557’	3,000
BP2	Katikati 1	Bay of Plenty	S 37° 33.365’	E 175° 54.397’	600
BP3	Katikati 2	Bay of Plenty			180
W1	Pukeatua 1	Waikato	S 38° 03.640’	E 175° 32.667’	2,250
W2	Pukeatua 2	Waikato			1,400
AK1	Huntly 1	South Auckland	S 37° 35.065’	E 175° 06.852’	260
AK2	Huntly 2	South Auckland			192
O1	Clinton	South Otago	S 46° 09.438’	E 169° 32.975’	7,500
SL1	Otautau	Southland	S 46° 06.972’	E 168° 01.748’	800
SL2	Papatotara 1	Southland	S 46° 09.395’	E 167° 36.358’	9,000
SL3	Papatotara 2	Southland			850
SL4	Fairfax 1	Southland	S 46° 11.538’	E168°02.422’	3,000
SL5	Fairfax 2	Southland			1,000

*Populations without coordinates were within 1 km of the site with the same name.

4.3 RESULTS

From the field surveys of *C. arvense* conducted in both the native (Europe) and introduced (NZ) ranges, 7 out of 12 populations contained rust in Europe, and 8 out of 13 populations contained rust in NZ. There was no significant difference between ranges in the mean percentage of diseased shoots (back-transformed means ± SE for EU = 0.78 ± 0.35 and NZ = 0.84 ± 0.34; deviance ratio = 0.02, *P*= 0.900; Fig. 4.1). There was also no significant difference between ranges in the mean percentage of shoots infected when comparing only diseased populations (back-transformed means ± SE for EU = 2.3 ± 0.69 and NZ = 1.7 ± 0.47; deviance ratio = 0.45; *P* = 0.517). The percentage of quadrats containing rust along the transect (for diseased populations only) was also not significantly different between ranges (back-transformed means ± SE for EU = 13.3 ± 3.0 and NZ = 17.0 ± 3.7; deviance ratio = 0.60; *P* = 0.458). When averaged over all rusted shoots, 97 percent were classified as "systemic" in Europe and 88 percent in NZ, and the small remaining proportions were classified as "localised" disease. In Europe, 12.4 percent of the *C. arvense* stems were attacked by various stem mining insects, but no such attack was found in any of the *C. arvense* surveyed in NZ.

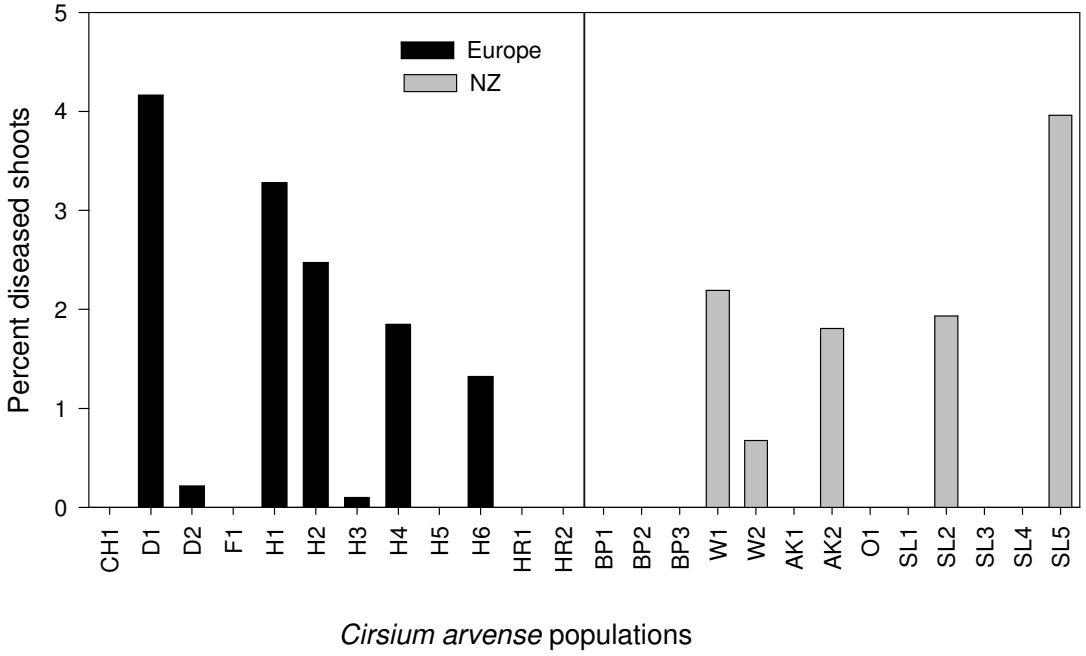

Figure 4.1. Percent *Cirsium arvense* shoots with rust disease out of the total number of shoots counted for each population. The populations BP1, BP2, SL4 (NZ), and H3 (Europe) had a low levels of rust that was not detected by the survey method.

4.4 DISCUSSION

Previously the proportion of rusted *C. arvense* shoots, and the amount of systemic vs. localised disease was unknown in NZ. Quantifying the level of rust in NZ was considered important for obtaining baseline data prior to the release of *C. onopordi*, so that the expected increase in damage to the thistle populations could be quantified. The surveys conducted in this study show that there is no difference in the level of rust between the native (Europe) and introduced (NZ) ranges of *C. arvense*. This is true when comparing the proportion of diseased shoots in the population and the number of quadrats containing rust along the population transect. It is also evident that there are no specialised insect herbivores feeding inside the stems of *C. arvense* in NZ. The monophagous stem-mining weevil, *Ceutorhynchus litura* (F.), was released in 1976, but failed to establish anywhere in NZ (Harman *et al.* 1996). Only two specialised insects are known on *C. arvense* in NZ: *Lema cyanella* (L.) and *Rhinocyllus conicus* (Frölich). *Lema cyanella* is a monophagous leaf chewing chrysomelid beetle that has been confirmed as established at only one site in NZ (Landcare Research, unpublished data). *Rhinocyllus conicus* is an oligophagous seed feeding weevil that was released for control of *Carduus nutans* L. (its preferred host) in NZ, but has also been found on *C. arvense* (Zwölfer & Harris 1984). This confirms the lack of establishment of biocontrol agents previously released for control of *C. arvense* in NZ noted by other authors (Harman *et al.* 1996; Julien & Griffiths, 1998). In NZ only five generalist insect herbivores have been reported in association with *C. arvense* (Spiller & Wise 1982), and the only insects commonly encountered on *C. arvense* in NZ are pollinators (M. Cripps, unpublished data). In essence, there are no stem mining insects in NZ that could vector the rust pathogen in the manner proposed by Friedli & Bacher (2001a). Since the incidence of rust disease is similar in both ranges, this raises the obvious question: how important are stem miners for vectoring this pathogen?

The idea that stem mining insects may increase systemic infection and disease is not new. In North America, there was speculation that the monophagous stem mining weevil, *Ceutorhynchus litura*, was increasing the level of rust (Peschken & Beecher 1973); however, it was later reported that this could not be substantiated (Peschken & Wilkinson 1981). Research indicating that *C. onopordi* is important for promoting systemic disease of *P. punctiformis* in *C. arvense* (Friedli & Bacher 2001a) contributed to interest in this weevil as a biological control agent in NZ, where the rust is already present. Friedli & Bacher (2001a) demonstrated that *C. onopordi* shows a preference for thistle shoots at an early stage of systemic disease when shoots are bearing pycnia and

emitting a characteristic floral odour. In Europe, the weevil by itself causes minimal impact on *C. arvense*; however, combined with the rust fungus the impact on *C. arvense* was believed to be synergistic (Friedli & Bacher 2001b).

Bacher & Friedli (2002) suggested that a mutualistic relationship exists between the weevil and the rust. They proposed that the fungus benefits from dispersal by the weevil, and the weevil benefits from increased fitness when developing on rusted shoots (Bacher *et al.* 2002). Since the weevil shows a distinct preference for diseased shoots, and healthy thistle shoots are a suboptimal host (Friedli & Bacher 2001a), there is an obvious quandary as to how new healthy shoots are infected. Bacher & Friedli (2002) address this issue and suggest that host finding ability is based on the relative proportion of healthy to diseased shoots in a population. At low frequencies of rust (below 23%) more healthy shoots should be attacked, in theory due to the weevil's poor host finding abilities (Bacher & Friedli, 2002; Moravie *et al.* 2006). Interestingly, the model of the dynamics among the weevil, rust and host plant does not consider the presence of the preferred host plant, *Cirsium vulgare* (Savi) Ten., which often co-occurs with *C. arvense* in both Europe and NZ (M. Cripps, personal observation).

The idea of a mutualistic interaction between herbivores and *P. punctiformis* is contrary to most knowledge about insects and biotrophic fungi. In general, *Puccinia* fungi are considered to be dispersed as windblown spores (Agrios 2005), and the interaction between insects and biotrophic fungi such as *Puccinia* is believed to be antagonistic (Hatcher 1995). This is because biotrophic fungi require living host plants for development. Thus, a herbivore that might weaken or accelerate the death of the host would be detrimental to the fungus. However, Friedli & Bacher (2001a) argue that if the insect spreads the pathogen more effectively, any fitness loss from the minimal impact of the insect will be off-set by the benefits of increased dispersal. The data presented here indicate that the rust fungus is similarly prevalent in Europe and NZ, indicating that a mutualistic relationship between the fungus and stem miners is not obligatory.

A further point of interest is the suggestion that urediniospores are the cause of systemic infection (Wandeler & Bacher 2006; Wandeler *et al.* 2008). In general, the life cycles of *Puccinia* fungi are fairly well understood (Scott & Charkravorty 1982; Agrios 2005). Dikaryotic urediniospores (n+n) develop into teliospores (2n) via the process of karyogamy. Germinating teliospores give rise to basidiospores (n), which cause infection, and subsequent production of monokaryotic pycniospores (n) – the characteristic spore stage indicative of systemic disease. Thus, it is unlikely that dikaryotic urediniospores –

by some other process – cause systemic infection and diseased shoots bearing monokaryotic pycniospores. Other authors have noted that some teliospores are always present in uredia (van den Ende *et al.* 1987); and, Wandeler & Bacher (2006) also report that a small proportion of teliospores were present in the inoculum carried by the weevils. Given the unlikelihood of urediniospores causing systemic infection, the teliospore contamination should not be excluded as a probable cause of the systemic disease observed in their studies.

We do not doubt that *C. onopordi* is capable of vectoring the rust, as are other specialised insects (Kluth *et al.* 2002). However, given that specialised insects do not exist on *C. arvense* in NZ (except *L. cyanella* on foliage and *R. conicus* in the seedheads) and that the rust is as common in NZ as it is in Europe, it is highly unlikely that insect vectors are necessary for transmission of this pathogen. If there were higher levels of systemic disease in Europe, this might indicate that the weevil is capable of increasing the incidence of systemic infection and disease. However, we have shown that the level of systemic disease is not different between ranges indicating that the weevil may not be necessary as a vector of this pathogen. It should be noted that our survey was based on only one year, and incidence of rust can vary from year to year according to environmental conditions (Scott & Charkravorty 1982). Furthermore, our surveys were carried out only once in each population, which would not capture any variation in the amount of rust, or the proportion of systemic vs. localised disease, from early to late season. Therefore, it is possible that the conditions during our survey season were not favourable for rust development in Europe, and that *C. onopordi* may be capable of increasing the level of rust during a season with more favourable environmental conditions. Genetic variation in clonal resistance/susceptibility to the rust pathogen is also reported to exist (Turner *et al.* 1981; Frantzen & Van der Zweerde 1994), and may also influence the level of rust observed in NZ. The data presented here indicate that stem mining vectors are not necessary for transmission of the rust pathogen, which may have implications for efforts to enhance biological control of *C. arvense*. However, the outcome of any interactions among *C. onopordi*, the rust, and *C. arvense* in NZ remains to be determined.

Chapter 5

Influence of natural enemies on *Cirsium arvense* in its native and introduced ranges

5.1 INTRODUCTION

It is well known that natural enemies (herbivores and pathogens) can cause extensive damage that reduces plant performance and fitness, but this does not necessarily mean that they regulate plant population dynamics (Crawley 1989). Whether or not natural enemies have a regulating influence on plant demography has been long debated (Hairston *et al.* 1960; Ehrlich & Birch 1967), and is still a pertinent issue in plant ecology today (Maron & Crone 2006). Understanding what regulates plant abundance and distribution is a fundamental ecological question, and has particular relevance to the application of classical biological control of weeds.

Classical biological control of weeds involves the importation of coevolved natural enemies from a plant's native range to its introduced range, and is based on the premise that natural enemies are capable of exerting a regulating influence on plant populations. Although the regulating ability of natural enemies has been largely untested, the enemy release hypothesis (ERH) is a common mechanism invoked to explain the apparent increased vigour of introduced invasive weeds (Elton 1958; Maron & Vilà 2001; Keane & Crawley 2002; Mitchell & Power 2003; Liu & Stiling 2006). Cases of successful classical biological control have provided evidence that in some circumstances natural enemies can regulate plant populations (reviewed by Harper 1977; Crawley 1989). In addition to controlling introduced invasive plants, some biological control agents have also been shown to have a regulating influence on non-target native plant species, most notably *Rhynocyllus conicus* (Fröl.), which has caused significant declines in populations of rare native thistle species in North America (Louda *et al.* 1997; Louda 1998; Louda *et al.* 2005; Rose *et al.* 2005). However, the majority of attempts at classical biological control of weeds have been unsuccessful (Julien & Griffiths 1998), which might indicate that natural enemies are generally not capable of regulating their host plant populations. Furthermore, cases of plant regulation by imported biological control agents are novel situations, since introduced natural enemies are in turn released from their own suite of predators and parasites, and thus regulation in the introduced range does not dictate regulation in the native range (Keane & Crawley 2002). A limited, but growing, number

of studies have investigated the affects of native natural enemies on native plants, and have found evidence that population growth of native plants can be controlled by specialised native natural enemies (e.g. Fagan *et al.* 2005; Rose *et al.* 2005; Jongejans *et al.* 2006; Miller *et al.* 2009). Investigating what controls plant populations in their native range may provide valuable insights into the ability of natural enemies to regulate plant populations and provide information on the potential effectiveness of classical biological control agents (Fowler *et al.* 1996; Scott 1996; Rees & Paynter 1998).

In New Zealand (NZ) there is renewed interest in classical biological control of *Cirsium arvense* (L.) Scop. As part of this biological control effort, a comparative survey of *C. arvense* was conducted in its native (Europe) and introduced (NZ) range, which showed that although plants experience reduced natural enemy pressure in NZ, plant performance was generally not different between ranges, and any differences detected could be attributed to more favourable environmental conditions in NZ (Chapter 2). Comparing the performance of a plant where it is native and introduced is important for gaining a more objective perspective on invasive weeds, and progressing beyond mere anecdotal accounts of increased vigour of exotic plants (Thébaud & Simberloff 2001; Hierro *et al.* 2005). However, it is difficult to attribute a causal mechanism to any measured differences or similarities of plant performance between its native and introduced range, and does not directly test the hypothesis that natural enemies have a regulating influence on plant performance, development, or population dynamics. Therefore, in an effort to examine the affects of natural enemies on *C. arvense*, we established experimental plots in the native (Europe) and introduced (NZ) ranges of the plant where natural enemies were excluded with the use of insecticide and fungicide applications, and compared with ambient natural enemy pressure in control plots.

Based on the ERH, we predicted (1) that in the native range, exclusion of insects or pathogens would have a positive effect, and that dual exclusion of insects and pathogens would have an increased positive effect on shoot performance, development, and population growth, relative to controls (ambient natural enemy pressure). Since natural enemy pressure is reduced in the introduced range we predicted (2) that exclusion of insects or pathogens and dual exclusion of both would not change shoot performance, development, or plant population growth, relative to controls.

5.2 METHODS

5.2.1 Study system

Cirsium arvense (L.) Scop. (Asteraceae) (Californian, Canada, or creeping thistle) is a perennial herb and a member of the tribe Cardueae, which comprises the plants commonly known as thistles (Bremer 1994). It is indigenous to Eurasia but has been accidently spread throughout temperate regions of the world, where it is considered one of the worst invasive weeds (Holm *et al.* 1977). It was first reported as an accidental introduction to NZ in 1878 (Kirk 1878), and is presently considered one of the worst weeds in NZ arable and pastoral production systems (Bourdôt & Kelly 1986; Bourdôt *et al.* 2007). *Cirsium arvense* spreads clonally by means of creeping lateral roots, and also reproduces by seeds (Fig. 5.1). *C. arvense* is almost completely dioecious with individual plants having capitula containing either male (staminate) or female (pistillate) florets. Seed production is dependent on insect pollination and proximity to male plants. Population sex ratios range from equal male:female ratios (Lloyd & Myall 1976) to extremely female biased sex ratios (Lalonde & Roitberg 1994). In general, few seeds are produced where male and female plants are separated by more than 50m (Lalonde & Roitberg 1994). In New Zealand, *C. arvense* flowers from December to February, and seeds are produced from December to April (Webb *et al.* 1988). In the native range of Europe, *C. arvense* flowers from July to September (Clapham *et al.* 1987), and seeds are also produced from July to September (M. Cripps, personal observation).

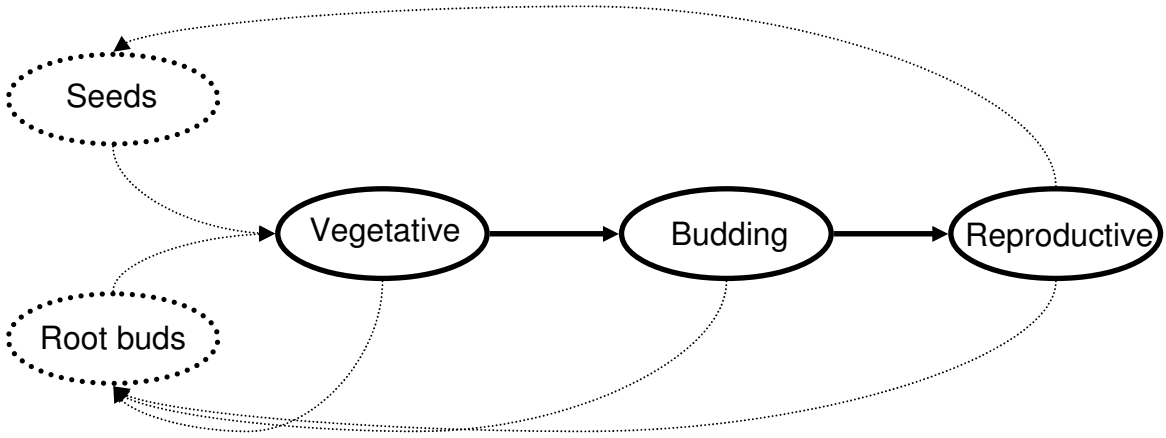

Figure 5.1. Schematic representation of the yearly life cycle of *Cirsium arvense*. New shoots can arise from either seeds or adventious shoot buds from the roots (root buds). Aerial shoot growth can contribute to the formation of new root buds. Growth and phenologies of the solid portions of the life cycle were monitored throughout the growing season of the plant. Dotted portions of the life cycle were not monitored in this study. New vegetative shoots can arise at any time during the growing season, and not all shoots necessarily transition from one phenological stage to the next.

There is a long history of control efforts against *C. arvense*, including cultural, chemical and biological methods (Donald 1990). In Europe many specialised (monophagous or oligophagous) insect herbivores are known to attack *C. arvense* (Zwölfer 1965) that could have a regulating influence on its populations. Classical biological control of *C. arvense* has been attempted in Canada, USA and NZ. In North America biological control of thistles in general was hampered by evidence that *R. conicus* was negatively impacting populations of related native thistles (Louda *et al.* 1997; Louda 1999). However, in NZ there are no native plants in the tribe Cardueae (Webb *et al.* 1988), enabling the recent release of two oligophagous thistle herbivores from Europe: *Cassida rubiginosa* Müller and *Ceratapion onopordi* Kirby. Previously, from 1979 to 1996, four insect herbivores [*Altica carduorum* Guér., *Hadroplontus litura* (F.), *Lema cyanella* (L.), and *Urophora cardui* (L.)] had been released in NZ for biological control of *C. arvense* (Julien & Griffiths 1998), but all have failed to establish (Harman *et al.* 1996), except for *L. cyanella*, which has reportedly established at one site in the North Island (Landcare Research, unpublished data). Additionally, *R. conicus* was released for control of *Carduus nutans* L. in 1973 (Julien & Griffiths 1998), and is known to also attack *C. arvense* (Zwölfer & Harris 1984). Other than insect herbivores, the highly specialized rust fungus, *Puccinia punctiformis* (Str.) Röhl., is also known to occur on *C. arvense* in NZ, and was present as early as 1881 (Cunningham 1927). Several generalist fungal pathogens are also known to attack *C. arvense* in NZ (Johnston 1990).

5.2.2 Natural enemy exclosure experiment

In Europe, experimental field plots were established in a pasture near St. Ursanne, Switzerland (N 47°22'00.93" E 7°08'09.30") that had been grazed previously by sheep and horses. The pasture was primarily composed of *Dactylis glomerata*, *Poa* sp. and *Lolium perenne*. Three species of *Cirsium* were also present in the pasture: *C. arvense*, *C. vulgare*, and *C. palustre*. Specialized natural enemies observed at the site included *Cassida rubiginosa*, *Ceratapion onopordi*, *Larinus* spp., tinged bugs, tephritid flies, plus the specialised rust pathogen, *P. punctiformis*. Generalist insect herbivores including spittle bugs, leafhoppers, aphids and thrips, were also frequently observed feeding on *C. arvense* at the field site. In NZ, two study sites were established, one near Lincoln and the other near Greenpark, Canterbury. Both sites were typical NZ pastures comprised primarily of *Lolium perenne* and *Trifolium repens*, and had been grazed previously by sheep. Both *C. arvense* and *C. vulgare* were present in both NZ field sites. In terms of

natural enemies observed at the NZ field sites, adults of *R. conicus* were observed at Lincoln, and *P. punctiformis* was present at both field sites. Other than these, only pollinating insects were observed visiting the flowers of the *C. arvense* shoots at the NZ sites. The areas containing the experimental plots in both Europe and NZ were fenced to exclude ungulate herbivores for the duration of each study season.

The experiment at each site was arranged in a 2x2 factorial randomized block design with four blocks. Within each block four 2x2m plots were marked with coloured stakes to indicate each of the four treatments (insecticide, fungicide, dual application, and control). In the centre of each plot, an internal $1m^2$ area was established wherein each shoot was individually tagged and numbered in order to monitor emergence, development and fate. For each tagged shoot in the internal quadrat, its phenology and height (cm), was recorded at each census (listed in Table 5.1). Newly emerged shoots were tagged and measured at each census.

The treatments were applied after the measurements were taken on each census date (Table 5.1). In each block three pesticide treatments (insecticide, fungicide, and dual application of insecticide + fungicide) plus a control (water) were applied. The insecticides used were imidacloprid (Confidor 70 WG applied at 1g/L) and bifenthrin (Talstar 80 SC applied at 0.5ml/L). The fungicides used were cholorothalonil (Bravo 500 SC applied at 3ml/L) and tebuconazol (Hornet 430 SC applied at 2.3ml/L). In the first study year at the Switzerland site the product formulation of tebuconazol used was Horizont 250 EW applied at 4ml/L, which was equivalent to the same amount of active ingredient applied the following year. Each entire 2x2m plot was sprayed with enough pesticide solution to give good plant coverage (i.e. 0.5L per plot). An equal amount of water was applied to the control plot in each block. Pesticide treatments were applied approximately every two weeks and alternated between systemic and contact modes of action from one census to the next (Table 5.1) in an attempt to maximise protection from natural enemies. Imidacloprid is a neonicotinoid insecticide with systemic activity, and particularly high efficacy against sucking insects (Mullins 1993). Bifenthrin is a contact pyrethroid insecticide that has long residual activity, does not break down easily in the sun and rain, and is effective at controlling a broad range of insects. Chlorothalonil is a contact fungicide and has protective activity against a broad range of fungi. Tebuconazole is a systemic fungicide with both protective and curative activity against a broad range of fungi.

Table 5.1. Dates of pesticide applications and censuses on *Cirsium arvense* plots in Europe (St. Ursanne, Switzerland), and New Zealand (Lincoln and Greenpark).

St. Ursanne 2007		St. Ursanne 2008		Lincoln 2007/08		Greenpark 2007/08	
Census date	Treatment	Census date	Treatment	Census date	Treatment	Census date	Treatment
26 May	Imidacloprid + Chlorothalonil	8 May	Imidacloprid + Chlorothalonil	8 November*	Imidacloprid + Chlorothalonil	13 November	Imidacloprid + Chlorothalonil
4 June*	Bifenthrin + Chlorothalonil	22 May	Bifenthrin + Tebuconazol	22 November	Bifenthrin + Tebuconazol	21 November	Bifenthrin + Tebuconazol
8 June	Imidacloprid + Tebuconazol	9 June	Imidacloprid + Chlorothalonil	6 December	Imidacloprid + Chlorothalonil	5 December	Imidacloprid + Chlorothalonil
20 June	Bifenthrin + Tebuconazol	22 June	Bifenthrin + Tebuconazol	20 December	Bifenthrin + Tebuconazol	19 December	Bifenthrin + Tebuconazol
12 July	Imidacloprid + Chlorothalonil	11 July	Imidacloprid + Chlorothalonil	10 January	Imidacloprid + Chlorothalonil	9 January	Imidacloprid + Chlorothalonil
26 July	Bifenthrin + Tebuconazol	29 July	Bifenthrin + Tebuconazol	24 January	Bifenthrin + Tebuconazol	23 January	Bifenthrin + Tebuconazol
15 August	Imidacloprid + Chlorothalonil	20 August	Imidacloprid + Chlorothalonil	11 February	Imidacloprid + Chlorothalonil	7 February	Imidacloprid + Chlorothalonil
31 August	Bifenthrin + Tebuconazol						
9 September	Imidacloprid						

*Pesticide treatments applied, but no shoot measurements taken.

In some circumstances pesticides can have phytotoxic or stimulatory effects on plant growth. Therefore, we conducted a potted-plant experiment from 1 November 2007 to 11 February 2009 in an outside garden enclosure at Lincoln University to examine for any pesticide effects on the growth of *C. arvense*. Plants were grown from root fragments (*ca*. 10cm length) planted in 9 litre plastic pots, and watered as needed. The experiment was set-up in a 2x2 factorial design with four blocks to reflect the field experiments. There were two potted plants for each treatment in each block (i.e. total of 32 potted *C. arvense* plants). The pesticide applications followed the same schedule as the NZ field experiments (Table 5.1) At the end of the growing period no significant main effects of insecticide or fungicide nor any interaction of these treatments on the number of shoots per plant, the maximum shoot height, or the proportion of shoots that transitioned to the reproductive growth stage (see Tables 5.2 and 5.3). Thus, we assume that the pesticides did not have an influence on plant growth in the experiments conducted in this study.

Table 5.2. Mean maximum shoot height and number of shoots of *Cirsium arvense* for each treatment (Control, Insecticide, Fungicide and Dual) in the Lincoln Garden experiment. The least significant difference value (LSD 5%) is given for comparison among the four means.

	Response variables	
Treatments	**Max. Height (cm)**	**Number of shoots**
Control	37.5	13.6
Insecticide	49.8	15.3
Fungicide	35.9	15.5
Dual	42.4	14.0
LSD (5%)	15.1	4.4

Table 5.3. ANOVA showing the main effects and interaction of insecticide and fungicide treatments on maximum shoot height and the number of shoots of *Cirsium arvense* in the Lincoln Garden experiment.

Response variable	**Source of variation**	**df**	***F***	***P***
Max. height (cm)	Insecticide	1	3.25	0.083
	Fungicide	1	0.75	0.395
	Insecticide x Fungicide	1	0.31	0.585
	Residual	31		
Number of shoots	Insecticide	1	0.00	0.967
	Fungicide	1	0.04	0.838
	Insecticide x Fungicide	1	1.07	0.310
	Residual	31		

5.2.3 Data analyses

Population flux (total shoots, natality, mortality and net change) was compared among the four treatments with a full factorial ANOVA at each census, testing for the two main effects of insecticide and fungicide application, and their interaction. Shoot natality included all new shoots that emerged after the initial cohort was labelled at the beginning of the experiment. Shoot mortality included all cohorts of shoots (i.e. initial shoots and subsequently emerged shoots). Net change was the difference between shoot natality and mortality. ANOVA was supplemented by unrestricted least significant difference (LSD 5%) comparisons among the four treatment means. Comparisons of shoot height and relative growth rate were compared in the same way for each census. Relative growth rate (RGR, cm day^{-1}) reflects the net change in shoot growth over a time period, and was calculated as $[\ln(H_1) - \ln(H_0)]/t$, where H_1 and H_0 are the mean shoot heights at two consecutive censuses (subsequent and previous, respectively), and t is the number of days between censuses (DeWalt *et al.* 2004).

The proportion of shoots from the initial labelled cohort that transitioned to the budding and reproductive (flowering and/or seeding) growth stages was analysed using a generalized linear model (GLM) with a logit-link function, allowing for over-dispersion and assuming a binomial distribution (with the binomial total being the total number of vegetative shoots in the initial cohort). A full factorial analysis was carried out to test the two main effects of insecticide and fungicide application, and their interaction, on the proportion of shoots that transitioned.

5.3 RESULTS

5.3.1 Shoot population flux

In NZ there were no significant main effects of insecticide or fungicide, nor any significant interaction of these treatment factors on the total number of shoots, shoot natality, mortality, and net change for all census dates at both field sites (Figs. 5.2 and 5.3). In Europe 2007, the total number of shoots was significantly less in the fungicide plots for the first two censuses (significant main effect of fungicide), but there were no significant main effects nor any interaction for the total number of shoots for the remainder of 2007, and no significant main effects nor interaction on the total number of shoots at any censuses in 2008 (Fig. 5.4A). In Europe 2007 and 2008, shoot natality was lowest in the control, but there were never any significant main effects of either insecticide or fungicide, nor any significant interaction of these treatment factors for shoot

natality in either year (Fig. 5.4B). Similarly, for shoot mortality in Europe 2007 and 2008, there were no significant main effects of either insecticide or fungicide, nor any interaction at any censuses in both years. In Europe 2007 there was a significant main effect of insecticide causing an increase in the net change in population growth at censuses six (day 82) and seven (day 98), but at census eight (day 112) the main effect of insecticide was not quite significant (F=4.64; P=0.060) (Fig. 5.4D). In Europe 2008, there was no significant effect of insecticide, but a significant main effect of fungicide causing an increase in population growth at censuses five (day 64), six (day 82), and seven (day 104) (Fig. 5.4D). There was never a significant interaction with the combination insecticide and fungicide on the net change in population growth. In Europe, net change in population growth became negative only in the control treatment for both years, with the exception of the insecticide treatment at the last census date in 2008 (Fig. 5.4D). However, in NZ net change in population growth became negative for all treatments at both field sites (Figs. 5.2D and 5.3D).

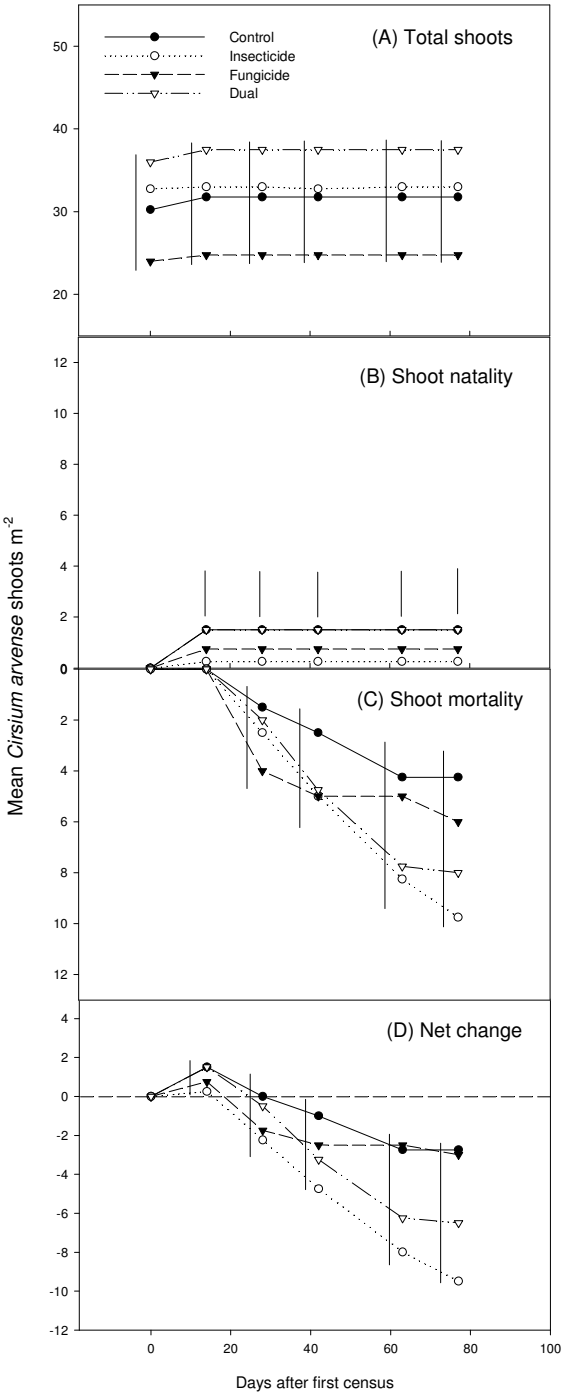

Figure 5.2. Mean population flux of *Cirsium arvense* (shoots m^{-2}) among four treatments (Control, Insecticide, Fungicide, and Dual) in Lincoln NZ, 2007/2008. (A) Mean cumulative total number of shoots for each treatment. (B) Mean cumulative shoot natality for each treatment. (C) Mean cumulative shoot mortality of all shoots for each treatment. (D) Mean cumulative net change (natality – mortality). Vertical bars preceding, or above the data points represents the LSD (5%) value for comparison among the four treatments.

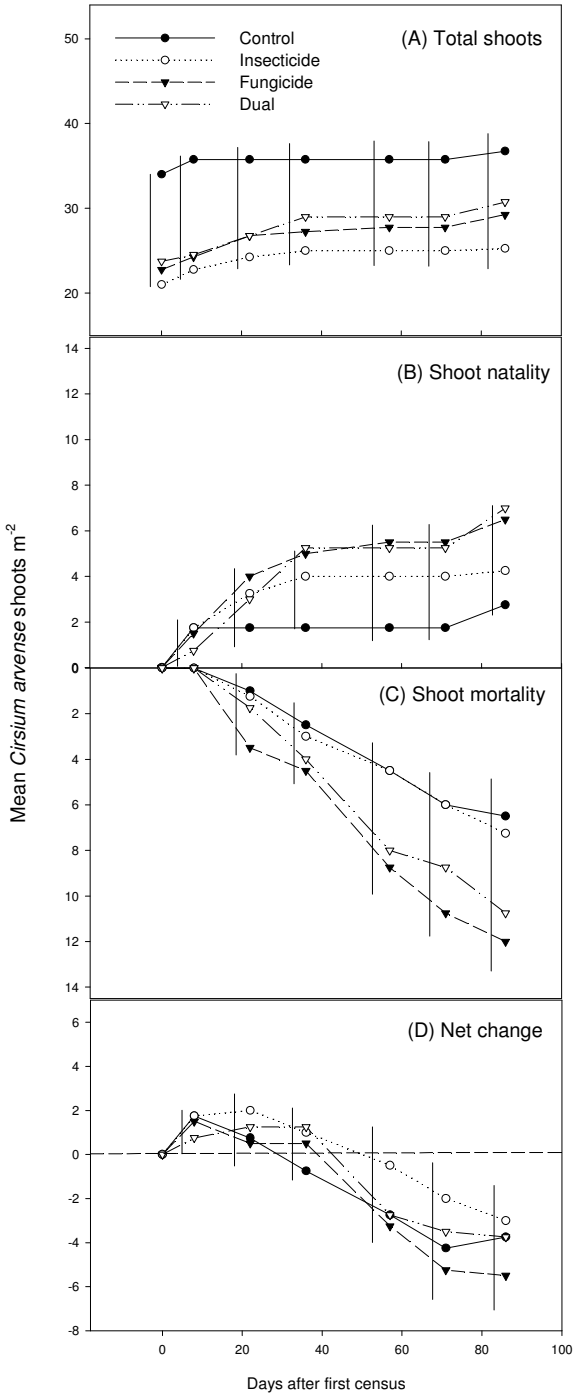

Figure 5.3. Mean population flux of *Cirsium arvense* (shoots m^{-2}) among four treatments (Control, Insecticide, Fungicide, and Dual) in Greenpark NZ, 2007/2008. (A) Mean cumulative total number of shoots for each treatment. (B) Mean cumulative shoot natality for each treatment. (C) Mean cumulative shoot mortality for each treatment. (D) Mean cumulative net change (natality – mortality). Vertical bars preceding the data points represents the LSD (5%) value for comparison among the four treatments.

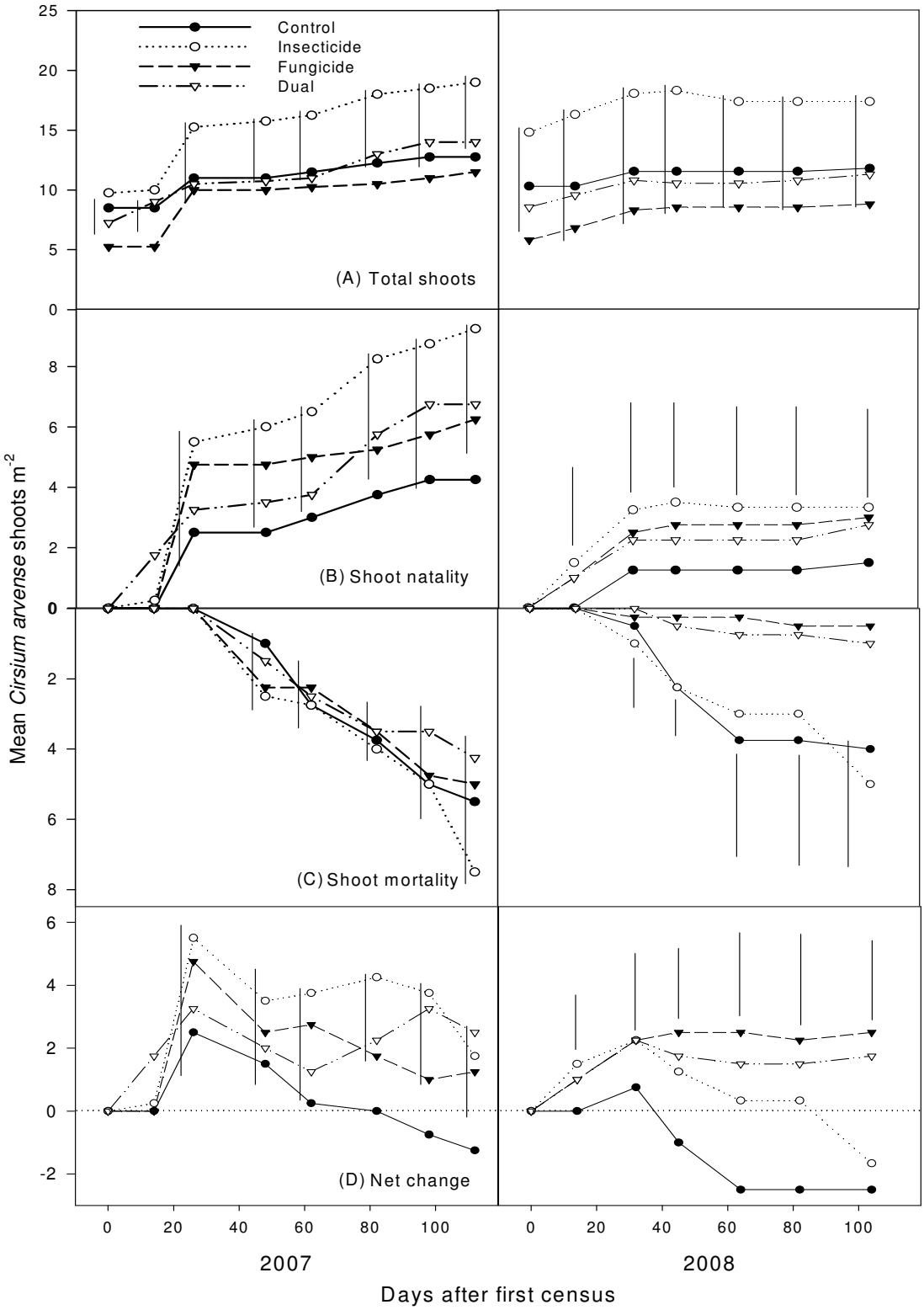

Figure 5.4. Mean population flux of *Cirsium arvense* (shoots m^{-2}) among four treatments (Control, Insecticide, Fungicide, and Dual) in St. Ursanne Switzerland, 2007 and 2008. (A) Mean cumulative total number of shoots for each treatment. (B) Mean cumulative shoot natality for each treatment. (C) Mean cumulative shoot mortality for each treatment. (D) Mean cumulative net change (natality – mortality). Vertical bars preceding, above or below the data points represents the LSD (5%) value for comparison among the four treatments.

5.3.2 Shoot height and its relative growth rate

In Lincoln shoot height was lowest in the control, but there were no significant main effects of insecticide or fungicide, nor any significant interaction of these treatment factors at any censuses at this field site (Fig. 5.5A). In Greenpark there was a significant main effect of insecticide, where shoots were significantly shorter in insecticide treated plots at every census except the last two (six and seven). There was also a significant main effect of fungicide at the Greenpark site where shoots in fungicide treated plots were significantly shorter at every census except the first one (Fig. 5.5B). There were no significant interactions at any censuses in Greenpark, except at census three, where shoots treated with both insecticide and fungicide were significantly shorter.

In Lincoln, there were no significant main effects of insecticide or fungicide, nor any interaction of these treatment factors on the relative growth rate (RGR) at any censuses (Fig. 5.5C). In Greenpark, there was a significant main effect of fungicide, where RGR was lower in the fungicide treated plots at the first two censuses, but there were no significant main effects for RGR at this site after census two, and never any significant interaction effects (Fig. 5.5D).

In Europe, shoot height tended to be greatest in the insecticide treatment, but was never significantly different from the control in both years (Fig. 5.6A,B). In Europe 2007, there was a significant main effect of insecticide starting at census three, and every census thereafter, where shoots were taller in insecticide treated plots. There was also a significant main effect of fungicide starting at census five, and every census thereafter, where shoots in fungicide treated plots were significantly shorter (Fig. 5.6A). In Europe 2008, there was a significant main effect of fungicide, where shoots were significantly shorter in fungicide treated plots at every census, but there was no significant main effect of insecticide treatment. There was never a significant interaction effect for shoot height in either year in Europe.

In Europe 2007, the only significant treatment effect on RGR was at census six, where shoots treated with fungicide had a significantly lower growth rate (Fig. 5.6C). In Europe 2008, the only significant treatment effect on RGR was at census three where shoots treated with insecticide had a significantly greater growth rate (Fig. 5.6D). There was never any significant interaction of the treatments on the RGR at any censuses in either year. In both NZ and Europe after approximately 60 days the shoot growth rate was close to zero (Figs. 5.5 and 5.6).

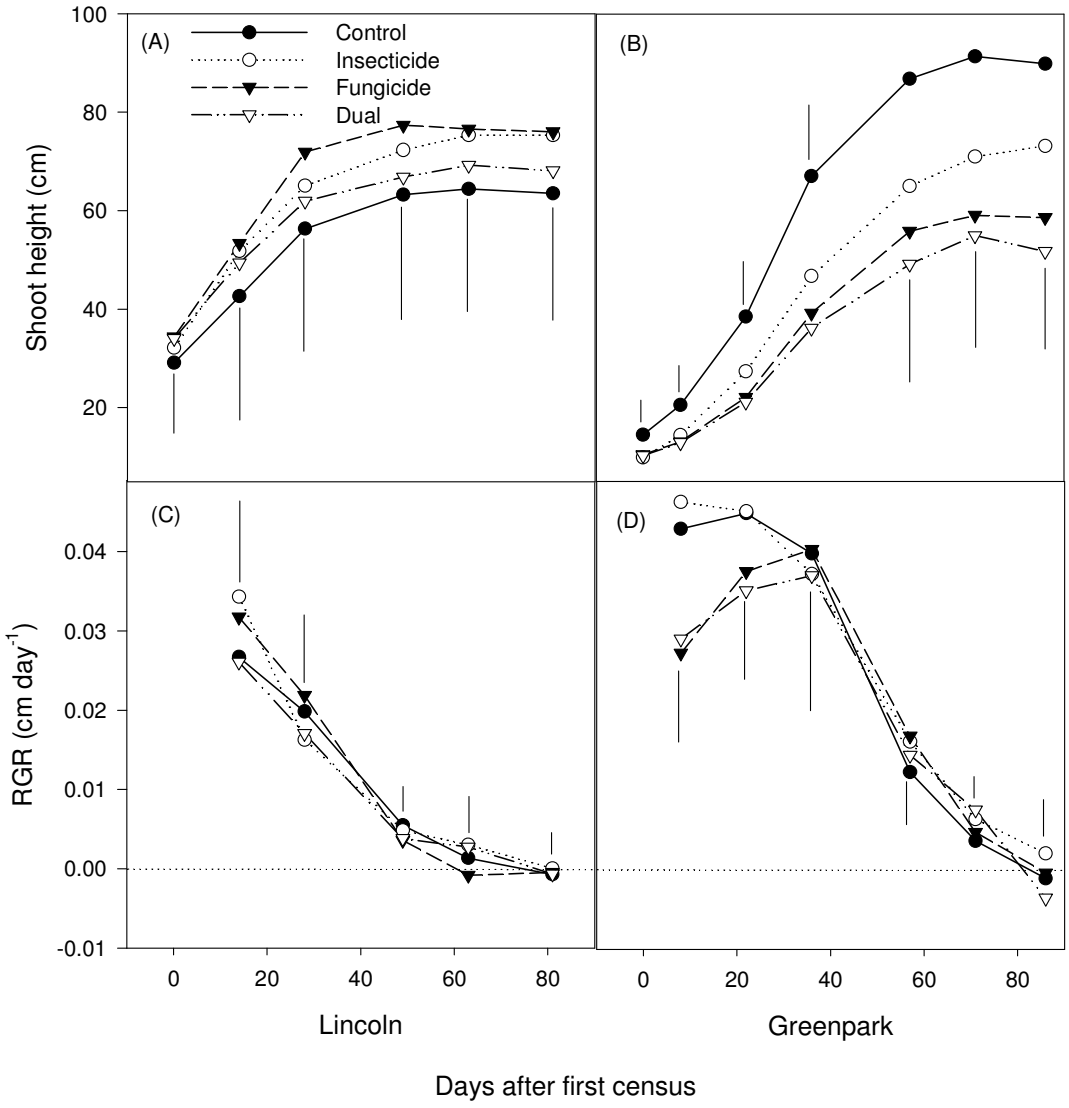

Figure 5.5. Mean shoot height (A, B) and relative growth rate of *Cirsium arvense* shoots (C, D) in Lincoln and Greenpark, Canterbury New Zealand, 2007/2008. Vertical bars above or below data points represent the LSD (5%) value for comparisons among the four treatments.

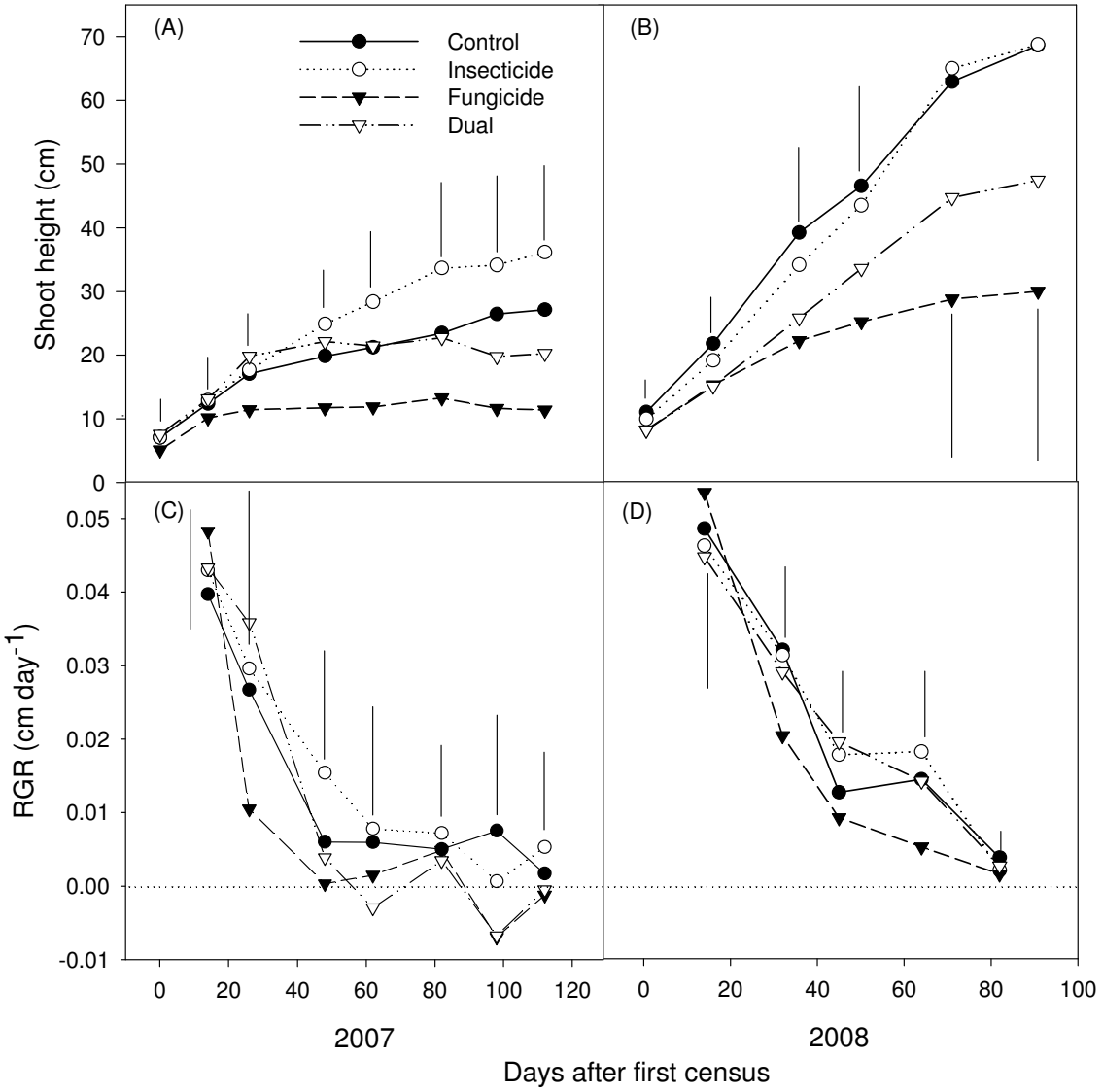

Figure 5.6. Mean shoot height and relative growth rate of *Cirsium arvense* shoots in St. Ursanne Swtizerland, 2007 and 2008. Vertical bars above or below data points represent the LSD (5%) value for comparisons among the four treatments.

5.3.3 Shoot phenology transitions

In Europe 2007, a significantly higher proportion of shoots transitioned from the vegetative to budding (deviance ratio = 23.8; $P<0.001$), and the budding to reproductive growth stage (deviance ratio = 9.03; $P=0.03$) when treated with insecticide (Fig. 5.7). In Europe 2008 there were no significant main effects of insecticide or fungicide on the proportion of shoots that transitioned from the vegetative to budding stage, but there was a significantly higher proportion of shoots that transitioned from the budding to reproductive stage when treated with insecticide (deviance ratio = 6.39; $P=0.045$). There

was never a significant main effect of fungicide treatment on the proportion of shoots that transitioned from one growth stage to the next in Europe in either year.

In Lincoln there were no significant main effects of insecticide or fungicide on any shoot transitions. However, in Greenpark significantly less shoots transitioned from the vegetative to budding stage when treated with fungicide (deviance ratio = 10.3; *P*=0.011); and significantly less shoots transitioned from the budding to reproductive growth stage when treated with insecticide (deviance ratio = 6.0; *P*=0.037) or fungicide (deviance ratio = 15.5; *P*=0.003). There were no significant interaction effects in either NZ or Europe.

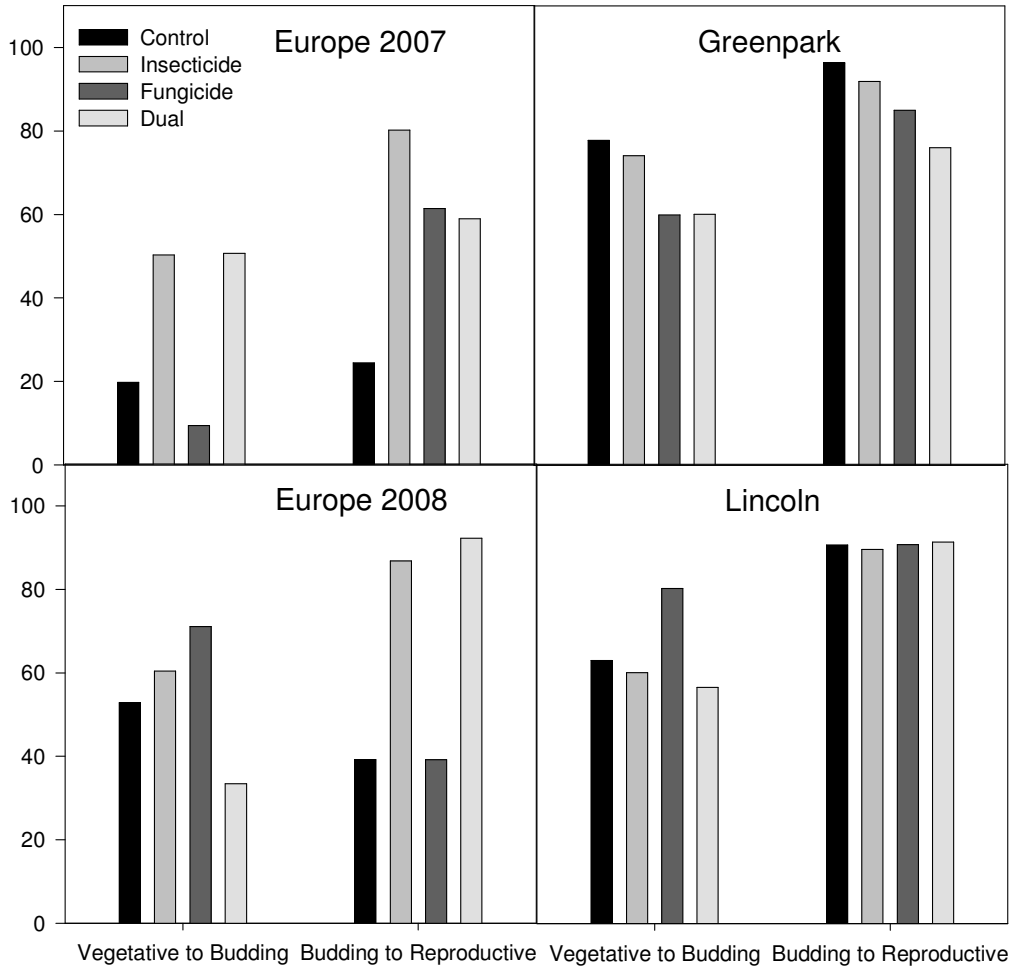

Figure 5.7. Mean proportion of vegetative shoots from the initial cohort that transitioned to the budding growth stage, and mean proportion of budding shoots that transitioned to the reproductive growth stage. Statistical inferences are based on logit transformed data.

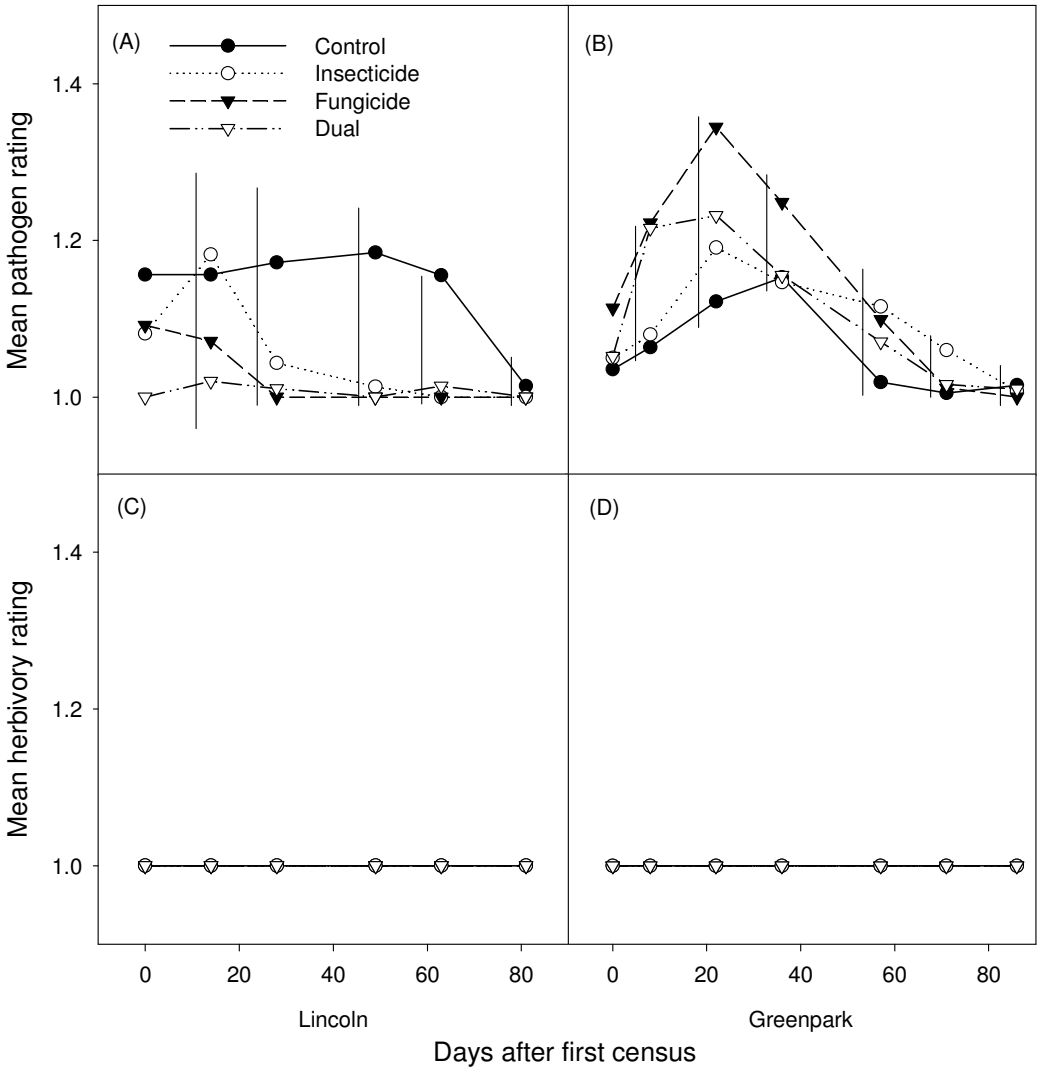

Figure 5.8. Mean damage ratings (1=no damage; 2=light; 3=moderate; 4=severe) from pathogens (A and B) and herbivores (C and D) on *Cirsium arvense* shoots in Lincoln and Greenpark, Canterbury New Zeland, 2007/2008. Vertical bars preceding data points represent the LSD (5%) value for comparisons among the four treatments.

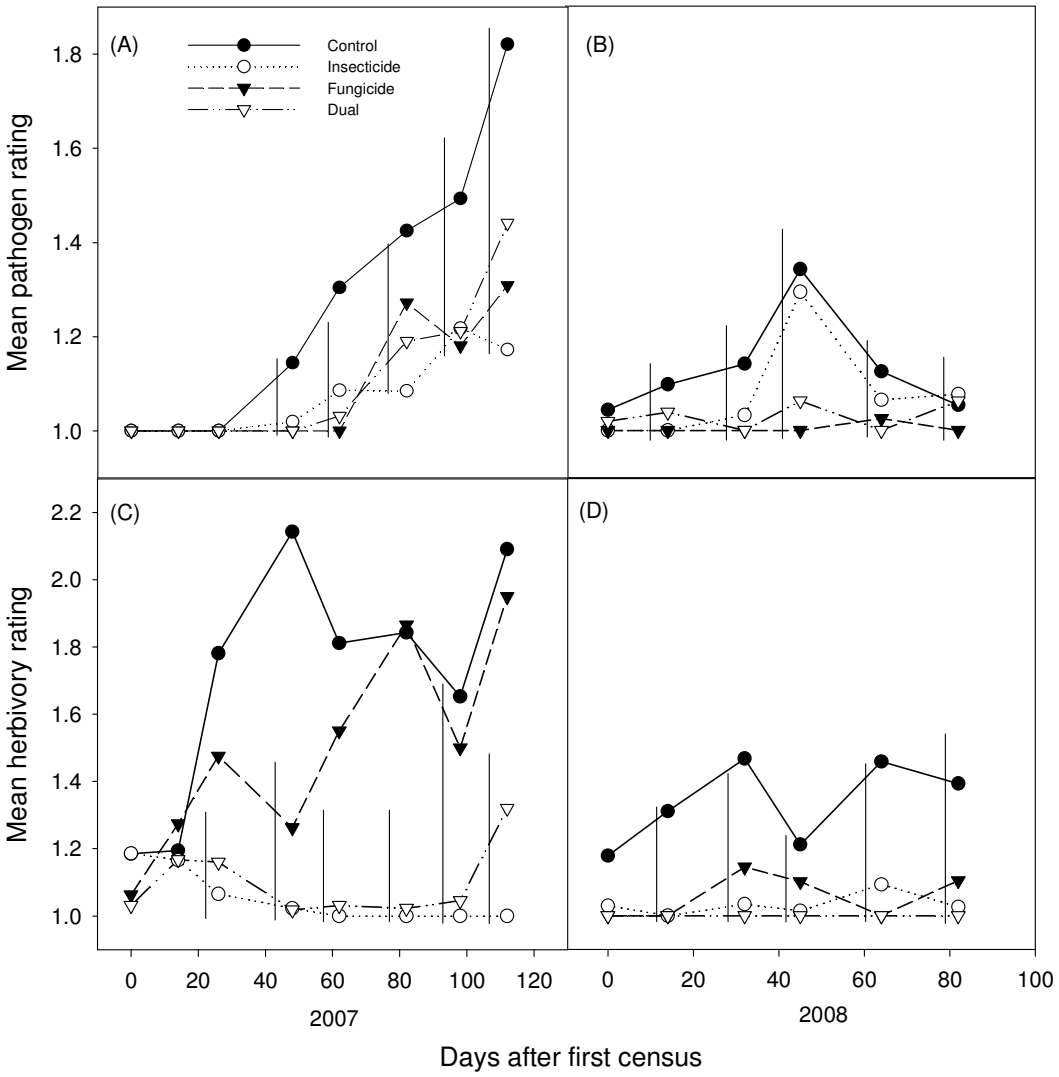

Figure 5.9. Mean damage ratings (1=no damage; 2=light; 3=moderate; 4=severe) from pathogens (A and B) and herbivores (C and D) on *Cirsium arvense* shoots in St. Ursanne Switzerland, 2007 and 2008. Vertical bars preceding data points represent the LSD (5%) value for comparisons among the four treatments.

5.3.4 Visual damage ratings

In NZ there was evidence of pathogen attack on *C. arvense*, but never any signs of external damage from herbivores, at both the Lincoln and Greenpark sites (Fig. 5.8). In Lincoln there were no significant effects of insecticide or fungicide treatment on visual rankings of pathogen damage at any census dates (Fig. 5.8A). In Greenpark there was a significant main effect of fungicide, where shoots had a greater average pathogen damage ranking at census two, but there were no significant effects of treatments at any other census dates (Fig. 5.8B).

In Europe, there was evidence of both pathogen and herbivore attack on *C. arvense* shoots (Fig. 5.9). There was no significant effect of insecticide or fungicide treatments on

the visual ranking of pathogen damage at any census dates in both 2007 and 2008 (Fig. 5.9A,B). In Europe 2007 there was a significant main effect of insecticide treatment, where shoots treated with insecticide had significantly reduced visual signs of herbivory. This effect started at census three and was consistent for every subsequent census date in 2007 (Fig. 5.9C). In Europe 2008 there was no significant effect of insecticide treatment (Fig. 5.9D). There was never a significant interaction effect of insecticide and fungicide treatment in either NZ or Europe.

5.4 DISCUSSION

5.4.1 Effects of natural enemy exclusion in the native and introduced ranges

With regard to our first hypothesis that exclusion of natural enemies in the native range would have a positive effect on plant growth, we found mixed evidence. Natural enemy exclusion had no effect on the performance of shoot height and relative growth rate of shoot height, but in some cases did have a positive effect on plant population growth and shoot development. Population growth remained positive in the native range where natural enemies were excluded, but went negative in the control where an ambient level of natural enemy pressure was present. However, not all exclusion treatments were always significantly greater than the control, and there was no consistent pattern in which enemy exclusion treatment had a greater affect on population growth compared to the control. With regard to shoot development, only exclusion of insect herbivory had a positive affect on transitions from the vegetative to budding and budding to reproductive growth stages. This effect on shoot development is also supported by the visual damage ratings, which indicated significantly reduced herbivory on insecticide treated plots in 2007. In 2008 there were no significant differences among the treatments in the visual ratings of herbivory, which corresponds well with the reduced impact on shoot transitions in that year. Therefore, studies from the native range indicate that population growth of *C. arvense* benefits from natural enemy release, and individual shoot development benefits more from insect herbivore release compared to release from pathogens. Our prediction that dual exclusion of herbivores and pathogens would have an increased positive effect was not supported.

In the native range the population growth of *C. arvense* was followed over two years. It has been hypothesised that reducing shoot biomass will cause a reduction in root biomass, and thereby reduce the number of shoot emerging the following season (Donald

1993; Bourdôt *et al.* 1998). Thus, we might have expected the total number of shoots to decrease where natural enemies were present, and possibly to increase where natural enemies were excluced, from one year to the next. However, there was no evidence of a year effect on the number of shoots in any of the treatments. It is possible that the ambient natural enemy pressure was not sufficient to reduce shoot biomass enough to cause a reduction in the overwintering root biomass. Precisely how much natural enemy pressure is necessary to cause an impact root biomass uncertain. Therefore, in the second year we conducted additional experiments with the biocontrol agent, *Cassida rubiginosa*, to test if realistic outbreak densities of this beetle might have an impact on *C. arvense* (Chapter 6).

In accordance with our second hypothesis that natural enemy exclusion would not affect plant growth in NZ we found no evidence that exclusion of insect herbivores or pathogens had an affect on plant growth, development or population growth, relative to controls. In the exotic range of a plant, we might expect natural enemies to have an impact if: (i) there are host shifts by native insects or pathogens, or (ii) if generalist insects and pathogens were able to utilize the plant, or (iii) if introduced biological control agents have established. The fact that insect exclusion had no effect is not surprising since it has been well documented that both specialist and generalist insect herbivory is almost completely absent on *C. arvense* in NZ (Spiller & Wise 1982; Fenner & Lee 2001; Chapter 3). However, in terms of pathogens, both specialist and generalist attack on *C. arvense* is present in NZ. A recent comparative survey showed that the specialised rust fungus, *P. punctiformis*, occurs at similar levels in NZ and Europe (Chapter 4; Cripps *et al.* 2009); and several generalist pathogens are also known to attack *C. arvense* in NZ (Cunningham 1927; Pennycook 1989; Waipara *et al.* 1993; Bithell & Stewart 2001). Shoots infected with *P. punctiformis* usually die before flowering (Watson & Keogh 1980; Thomas *et al.* 1994), but even artificial manipulation with this pathogen has failed to have population level impacts on *C. arvense* (Kluth *et al.* 2003). Inundative treatment with the generalist pathogen *Sclerotinia sclerotiorum* (Lib) de Bary has been shown to have detrimental effects on the population growth of *C. arvense* in NZ (Bourdôt *et al.* 1995; Bourdôt *et al.* 2006), which suggest that under some circumstances (e.g. epidemic conditions) generalist pathogens might be able to have a regulating influence on the demography of *C. arvense*. Nevertheless, in this study, the exclusion of pathogens with the use of fungicides had no affect on *C. arvense* in NZ, which might point towards a

greater regulating influence of herbivores, since natural enemies did have an effect in the native range.

Similar to our study, DeWalt *et al.* (2004) investigated the affects of natural enemies on *Clidemia hirta* in its native and introduced ranges with the use of pesticide exclosure experiments. DeWalt *et al.* (2004) did not investigate the impact of natural enemies on population growth of *C. hirta*, but did find that exclusion of natural enemies had a positive effect on growth and survival in its native range, but no effect in its introduced range, thus providing supportive evidence for the ERH. They found that insecticide or fungicide applied alone had a positive affect on *C. hirta* growth and survival, and that the dual application of both had an additive positive affect on plant growth and survival. Similarly, we also found that exclusion of natural enemies benefited *C. arvense* shoot development in the native range, but in contrast, this was only evident with insecticide treatment, and not with the fungicide or dual treatment.

Probably the most extensively studied invasive weed in its native and exotic ranges is broom, *Cytisus scoparius* (Fowler *et al.* 1996; Paynter *et al.* 1998; Memmott *et al.* 2000; Paynter *et al.* 2000; Sheppard *et al.* 2002; Paynter *et al.* 2003). In the case of broom, there was no clear signal that natural enemies were a key population regulating factor for the species in the native range, but their influence could not be ruled out (Paynter *et al.* 2000; Paynter *et al.* 2003). Exclusion of natural enemies in its native range with the use of pesticides had no effect on the survival, growth, reproductive age, or number of seeds produced per pod; but rather disturbance appeared to be the most important factor contributing to the increase in these traits of broom in its native range (Paynter *et al.* 1998). However, earlier research in the native range of broom showed that long-term exclusion of natural enemies had significant affects on plant survival, size and vigour (Waloff & Richards 1977), which in theory should translate into reduced population growth of broom (Rees & Paynter 1997). In the introduced range of NZ an insect exclusion study with broom indicated that insect herbivores had a significant impact on broom growth and both native insects and introduced biological control agents were implicated in the effect (Syrett *et al.* 1999).

5.4.2 Pasture competition and enemy release

Interspecific plant competition and attack from natural enemies are often interacting forces that contribute to the regulation of plant populations (del-Val & Crawley 2005). Of particular interest in this study was the importance of natural enemies, although teasing

apart the effects of competition and natural enemy attack is difficult (Crawley 1983). An important aspect to consider in this experiment is that the entire exclusion plots in the pasture were sprayed with pesticide treatments, thereby also releasing competing vegetation from natural enemy attack. If competing vegetation experienced a similar affect from the exclusion treatments as *C. arvense* then we would still expect plant performance to be lower overall in the control plots compared to the pesticide treated plots. However, we cannot be certain if release from natural enemies had a disproportionate effect among the competing plants that could have concealed the effect of enemy release on *C. arvense*.

Excluding ungulate grazers allowed us to more specifically examine the affects of specialised natural enemies. However, the typical habitat of *C. arvense* in both its native and introduced ranges is in pasture where grazing livestock preferentially feed on more palatable forage plants such as grasses and clovers, which allow *C. arvense* to grow free of interspecific competition (Bourdôt & Kelly 1986; Scholz 1995). Evidence would suggest that these more palatable species should be stronger competitors in the absence of grazing herbivores (Rees *et al.* 2001), and therefore the effect of strong interspecific plant competition may have also concealed affects of specialised natural enemies on *C. arvense*. In general, new shoot recruitment was minimal in both the native and introduced range of our study, which was likely due to the competing vegetation and the lack of disturbance from ungulate grazers that can create microsites which are conducive for new shoot growth (Edwards *et al.* 2000). Nevertheless, in our study we have detected evidence supporting the idea that *C. arvense* benefits from natural enemy release, but it is possible that the affects of specialised natural enemies would be stronger in a typical livestock grazed pasture.

In NZ, the competing vegetation in pastures is also introduced from Europe, and thus the interspecific plant competition experienced by *C. arvense* in NZ is likely similar to in its native range. This was demonstrated with the invasive weed, *Echium plantagineum*, where pasture competition was shown to be equivalent between its native range of Europe and introduced range of Australia (Grigulis *et al.* 2001). Therefore, we would not expect the exclusion of natural enemies to differentially favour the competing vegetation in NZ, and we believe the results are consistent with the prediction that natural enemy pressure is not sufficient enough in NZ to have an influence on *C. arvense*.

5.4.3 Potential regulation of *Cirsium arvense* by natural enmies

A recent comparative survey showed that performance of *C. arvense* was generally not different in Europe and NZ, and therefore the importance of natural enemies in regulating the plant was doubted (Chapter 3). However, the survey did not consider population growth, and shoot development of the plant. The data presented here suggest that although plant performance is similar in the native and introduced ranges, natural enemies may have a regulating impact on the population dynamics, and thus provides support for the ERH. However, it is difficult to identify which natural enemies might be causing these effects. In the introduced range of NZ, insect herbivory is virtually absent, but specialist and generalist pathogen attack is present. Since exclusion of pathogens in NZ had no significant effect, the data point toward a greater importance of insect herbivory. If generalist insect herbivory in NZ was sufficiently similar to that in the native range, we might be able to infer that generalist herbivores were not as important as specialist herbivores. However, it appears that even generalist herbivory is reduced in NZ, and therefore the relative importance of generalist vs specialist insect herbivores on *C. arvense* remains uncertain. Nevertheless, the indication that natural enemies have an impact on the population dynamics of *C. arvense* in its native range is promising for the prospect of classical biological control in NZ. Even if there were no evidence that natural enemies influenced the growth of *C. arvense* in the native range, there would still be potential for introduced biological control agents to have an influence on the plant in NZ. For instance, in the case of broom there was mixed evidence pertaining to the impact of natural enemies in its native range, but in the introduced range of NZ, an imported biological control agent was reported to occur at comparatively increased abundance, and to have an impact on broom growth when compared to plants where insects were excluded with an insecticide (Memmott *et al.* 1997). Thus, the response of an imported biological control agent released into a novel environment, where it is released from its own biotic constraints, is difficult to predict, and may have unexpected impacts on a plant in its exotic range. The recent releases of *C. onopordi* and *C. rubiginosa* in NZ have potential to impact the demography of *C. arvense*, and follow-up studies would provide valuable information on their effectiveness.

Chapter 6

Effects of pasture-competition and specialist herbivory on the performance of *Cirsium arvense*

6.1 INTRODUCTION

Competition and herbivory are both forces that can limit plant growth and abundance (Crawley 1983; Tilman 1988). Understanding how to manipulate these forces in order to enhance weed control is an important element of biological control research. In pasture systems, preferential grazing on desirable forage species by livestock can alter the competitive balance in favour of weed species, and be the cause of weed infestations (Sindel 2006). Introduction of a biological control agent that specialises on the weed species may adjust the competitive balance in favour of the forage plants. Substantial research has been conducted on pasture management that increases the competitive ability of forage plants for the benefit of controlling weeds (see Campbell 1997; Dowling *et al.* 2000). However, comparatively less is known about how specialised insect herbivores act in concert with interspecific plant competition (Sheppard 1996).

In a review of studies that investigated the impact of competition combined with herbivory from biological control agents, Sheppard (1996) indentified three categories of possible outcomes: "substitutive", "additive" (or multiplicative), and "synergistic". A substitutive result is where one factor completely suppresses any impact of the other factor. An additive result is where both factors have an impact, but without an interaction. Finally, a synergistic result is where the combined factors interact to have a greater impact than the sum of their individual effects. From a weed control perspective, synergistic outcomes resulting from biological control agents would be the best scenario. However, the majority of studies investigating the combined effects of herbivory and interspecific competition have found an additive type of relationship, with competition usually being a more dominant factor (Sheppard 1996).

Cirsium arvense (L.) Scop. (Asteraceae) is a perennial herb indigenous to Eurasia that has been inadvertently transported to temperate regions of the world where it is considered one of the worst weeds of arable and pastoral production systems (Holm *et al.* 1977; Donald 1990; Skinner *et al.* 2000). In New Zealand (NZ), *C. arvense* is also one of the worst weeds of pastoral systems (Bourdôt & Kelly 1986; Bourdôt *et al.* 2007). There has been a long history of control efforts against *C. arvense* in NZ pastures, including

chemical (Meeklah & Mitchell 1984), altering grazing management (Hartley *et al.* 1984; Edwards *et al.* 2000; 2005), and classical biological control (Jessep 1989).

Previously, from 1979 to 1996, four insect herbivores [*Altica carduorum* Guér., *Hadroplontus litura* (F.), *Lema cyanella* (L.), and *Urophora cardui* (L.)] had been released in NZ for biological control of *C. arvense* (Julien & Griffiths 1998), but all have failed to establish (Harman *et al.* 1996), except for *L. cyanella*, which has reportedly established at one site in the North Island (Landcare Research, unpublished data). However, recently there has been renewed interest in classical biological control of *C. arvense* in NZ, which has resulted in the first releases of the leaf-feeding beetle, *Cassida rubiginosa* Müller (hereafter referred to as *Cassida*), in spring 2007 (ERMA-NZ 2007). This foliage feeding beetle is indigenous to Eurasia. It is an oligophagous feeder on plants in the tribe Cardueae, commonly known as thistles, but shows pereference for *C. arvense* (Zwölfer & Eichhorn 1966). In its native range, and introduced range of North America, *Cassida* is univoltine (Zwölfer & Eichhorn 1966; Ward & Pienkowski 1978a). Development time from egg to adult is highly temperature dependent, with no development occurring at temperatures below 10°C (Ward & Pienkowski 1978a). Overwintered adults emerge in early spring and typically deposit egg masses (oötheca) on the underside of thistle leaves. Oviposition is most prolific in early spring, then slowly diminishes, and ceases by mid summer. Larval development progresses through five instars before pupation, which occurs on the soil surface. Second generation adults emerge in mid to late summer and have a brief feeding period on thistle foliage before overwintering.

Pasture grasses are known to be highly competitive against *C. arvense* (Bourdôt 1996). Thus, the potential impact that this beetle might have on *C. arvense* in different competitive scenarios typical of NZ pastures was of particular interest for improving biological control of this weed. Therefore, we conducted an outdoor potted-plant experiment (Garden experiment) with low and high densities of *Cassida* larvae combined with different levels of interspecific competition from typical NZ pasture species (i.e. perennial ryegrass and white clover). Different levels of competition were created by simulating selective grazing that would occur in a typical sheep or cattle grazed pasture in NZ (Cosgrove & Edwards 2007). In addition we carried out a field experiment (Field experiment) at a natural *C. arvense* site with grass competition, releasing high densities of *Cassida* to quantify *Cassida* impact under more natural conditions. The experiments were conducted in the native range of the plant in Europe where an ample supply of *Cassida*

beetles could be assured. Our specific hypotheses were: (1) that the different simulated grazing treatments would alter the competitive balance so that ungrazed treatments would reduce *C. arvense* performance more than grazed treatments and long-grazed treatments would reduce *C. arvense* performance more than short-grazed treatments. Similarly, we hypothesized (2) that herbivory by *Cassida* would reduce the performance of *C. arvense*, and that high larval densities would have a greater effect than low densities. We also hypothesized (3) that the interaction of competition and *Cassida* would be additive, with both factors contributing to decrease the performance of *C. arvense*. Finally, we hypothesized (4) that a realistic high density of *Cassida* larvae would reduce the growth and development of *C. arvense* in an open field situation.

6.2 METHODS

6.2.1 Rearing of *Cassida*

In order to ensure a sufficient number of beetle larvae for both experiments, a colony of *Cassida* beetles was established at CABI Europe-Switzerland (CABI-E-CH) from adult beetles collected on *C. arvense* and *Cirsium vulgare* (Savi) Ten. from 24 April to 1 May 2008 in Fessenheim France and Müntschemier Switzerland. Adult beetles were kept on covered potted *C. arvense* plants, and egg masses were regularly collected off the plants. Egg masses were placed in Petri dishes on moist filter paper, and when larvae hatched they were transferred to *C. arvense* shoot cuttings placed in plastic boxes that were housed in an outdoor enclosure.

6.2.2 Garden experiment

In spring 2008, an experiment was established in the garden of CABI-E-CH in Delémont, Switzerland (47°21' N, 7°22' E) to assess the impact of *Cassida* on *C. arvense* combined with different levels of interspecific plant competition. Seeds of *C. arvense* were obtained from the Nantes Mairie Jardin Botanique, France. On 11 March, seeds were sown into trays (18 x 13 cm x 6 cm deep), and placed in a heated greenhouse for germination. After development of the first true leaves, 48 individual seedlings were transferred into plug trays (5 cm diameter, 5 cm deep), and kept at 10°C in order to slow growth until being used in the experiment. The experiment was set up in a randomised 4x3 factorial design with four blocks. The two factors were plant competition (4 levels) and herbivory (3 levels).

Plant competition consisted of a mixture of perennial ryegrass (*Lolium perenne* L.) (87% by seed weight) and white clover (*Trifolium repens* L.) (13% by seed weight), which are the typical forage plants in NZ pastures (Daly 1973). These seed weight proportions are also typical for this type of pasture sown in NZ (Fleming 1996). Seeds of perennial ryegrass and white clover were purchased from a local commercial supplier (Landi, Switzerland). On 28 April 0.4g of seed mixture was sown into each of 48 circular pots (33 cm diameter, 15 L volume) corresponding to 46 kg of grass/clover mixture per ha. Seeds were sown into pots filled with a mixture of turf-based garden soil (Florabella, 150-300 mg N l^{-1}), sand and vermiculite. The experiment was set-up on a section of mown lawn in the CABI-E-CH garden. All pots were placed on weed mats to prevent other plants from growing between the pots in the experiment, and to prevent escape of *C. arvense* roots from the base of the pots. The plant competition factor consisted of four levels: no competition, short-clipped, long-clipped, and unclipped. The different levels of clipping simulated "short grazing" typical of sheep, and "long grazing" typical of cattle (Cosgrove & Edwards 2007). When the grass/clover mixture reached 6 cm in the "short grazed" treatment it was clipped down to 2 cm; and when the grass/clover mixture reached 15 cm in the "long grazed" treatment is was clipped down to 10 cm. Clipping treatments were applied approximately twice per week. Thus, the clipping treatments simulated a selective grazing by either sheep or cattle.

On 13 May, a single *C. arvense* seedling (3 to 4 leaves) was transplanted into the centre of each of the 48 pots in the experiment. At the time of transplanting, the grass seedlings had one to two leaves, and the clover seedlings were at the cotyledon to one true leaf stage. All pots were watered regularly as needed for the duration of the experiment. Molluscicide pellets (3.5% Metaldehyd, Limax Syngenta Agro) were placed around the base of the pots to control slugs.

The beetle herbivory factor consisted of three levels: nil (no larvae), low (5 larvae), and high (20 larvae). The beetle densities corresponded to realistic low and high population densities of beetle larvae per shoot observed in natural field populations of *C. arvense* in Europe (Bacher & Schwab 2000; M. Cripps, personal observation). On 12 June, first to second instar larvae were added to the pots receiving the herbivory treatment (eight pots per block with larvae added). All pots in the experiment (including beetle-free treatments) were covered with gauze mesh bags to prevent larvae from escaping and to protect them from predators. The longest possible diameter of each *C. arvense* rosette was measured just prior to release of the beetle larvae. As a check on the initial allocation of

plants to treatments, there were no significant main effects of competition or *Cassida* nor any interaction between the two treatment factors on the diameter of *C. arvense* plants at the time of *Cassida* larvae release. When initial plant diameter was used as a covariate, it was not significant for any of the plant parameters measured at the end of the experiment. On 19 June, all pots to which beetle larvae had been added were carefully examined to assess the survival of the larvae. If larvae were found to be missing, additional first to second-instar larvae were added in order to maintain the desired density for the treatment.

Over a six-day period from 31 July to 5 August 2008, *C. arvense* plants from the experiment were harvested. All aerial shoots were cut at the soil surface level, the shoots were counted, and their basal diameter and height was recorded. The subterranean shoots and roots of *C. arvense* were separated from the soil and competing plants if present. The subterranean shoots (> 4mm length) were then counted and separated from the roots. The aerial shoots, subterranean shoots, and roots from each pot were placed in a drying oven at 70°C for approximately 48 hrs and their dry weights were measured. The fresh weight of root samples was recorded prior to drying.

To obtain an estimate of the number of root buds, a 4.0g fresh weight subsample of lateral root was taken from each pot of blocks 1 to 3. Root buds (adventitious buds arising from root tissue) were defined as all visible emerged buds 4mm in length or less plus all buds that had not yet emerged through the root cortex. In order to also count the number of unemerged buds, the root subsample was placed in a test tube with a solution of 80% lactic acid. The test tubes containing the roots and lactic acid solution were kept in a hot water bath at 55 to 60°C for one week in order to dissolve the cortex and make unemerged buds visible (Nadeau & Vanden Born 1989). After the root cortex was dissolved, the roots were removed, rinsed with water, and the number of buds per sample was counted using a microscope at 10x magnification. To allow conversion to the number of root buds per g of root dry weight the proportion of root dry matter was calculated [dry weight / (fresh weight - 4g)] for each sample.

6.2.3 Field experiment

In spring and summer 2008, an experiment was conducted in a natural field population of *C. arvense* near St. Ursanne Switzerland (N 47°22'00.93" E 7°08'09.30") that had been previously grazed by sheep and horses. The *C. arvense* population was non-contiguous, and consisted of five distinct patches that occupied an area with a perimeter of approximately 80 m. The competiting vegetation in the study area consisted primarily of

the grasses *Dactylis glomerata*, *Poa* sp. and *Lolium perenne*. The entire study area was fenced to exclude ungulate grazers for the duration of the experiment.

Another experiment testing the exclusion of natural enemies was also being conducted (Cripps, unpublished data) in the same study area, of which the control plots (ambient natural enemies) were used for comparison with the *Cassida* release treatment., The *Cassida* release plots were added into each of the four blocks of plots already set-up in the study area and a fifth plot was established separately within the same study population. All plots were 1 m^2 and every shoot within each plot was labelled. The phenology, height and base diameter of each labelled shoot was monitored over five censuses. The first plant census was carried out on 22 May 2008 for the control plots, and 27 May for the plots where *Cassida* was to be released. Subsequent censuses were carried out on 9 June, 22 June, 11 July and 29 July 2008 for both the control and *Cassida* plots. The *Cassida* release treatment was applied on 27 May 2008 by adding first to second-instar larvae to each shoot in the five "*Cassida* plots": 20 larvae for each shoot 15 cm or taller and 10 larvae per shoot for shoots less than 15 cm in height. On 4 and 18 June densities of the *Cassida* larvae were assessed and additional larvae were added if necessary in order to maintain the desired beetle density according to the shoot height.

6.2.4 Data analyses

For the garden experiment, a full factorial ANOVA was carried out to test the two main effects of competition and *Cassida*, and their interaction. This analysis included examination of the orthogonal contrasts within each of the two treatment factors and the corresponding single-degree-of-freedom interaction contrasts (listed in Table 5.1). All dry weights and the number of root buds were log-transformed and the number of aerial and subterranean shoots were square-root transformed in order to homogenise the variances. Three gross outliers were present in the dry weight data due to zero or one shoot per plant. These three values were omitted from each of the dry weight variables in order to meet the underlying assumptions of the analysis.

For each census date in the Field experiment, two treatments (control and *Cassida*) were compared using ANOVA for the mean shoot height, mean shoot base diameter (SBD), and mean shoot volume. Shoot volume was calculated as π $(SBD/2)^2$ x height, and was cube-root transformed in order to homogenise the variances. Shoot volume was found to be a good predictor of shoot dry weight ($y=0.49x$; $R^2=0.90$; M. Cripps, unpublished data). The proportion of shoots that transitioned to the reproductive

(flowering and/or fruiting) growth stage in the Field experiment was analysed using a generalized linear model (GLM) with a logit-link function, allowing for over-dispersion and assuming a binomial distribution (with the binomial total being the total number of vegetative shoots in the initial cohort). The same analysis was also conducted using initial shoot height or initial shoot volume as a covariate, which resulted in a logistic curve being fitted to the observed data for each treatment.

6.3 RESULTS

6.3.1 Garden experiment

Interspecific plant competition caused a significant reduction in all measured parameters of *C. arvense* except mean shoot base diameter and mean shoot height, and mean number of root buds per gram dry weight (Table 6.1). Class comparison contrasts between different groups of competition treatments revealed that some *C. arvense* parameters were affected more by competition than others (Table 6.1; Fig. 6.1). The number and DW (dry weight) of aerial shoots were the only variables that were reduced with each incremental level of competition (Table 6.1; Fig. 6.1 A,C). The unclipped level of competition caused a significant reduction in all *C. arvense* parameters measured (except mean shoot base diameter and shoot height) compared to the clipped competition. Mean shoot base diameter and shoot height in the unclipped treatments were significantly increased due to a lower number of total shoots (i.e. few new small shoots emerging with high competition). The short vs. long clipped competition levels caused a significant reduction in the number and DW of aerial shoots, but had no significant effect on any other variables (Table 6.1).

The presence of *Cassida* caused a reduction in root dry weight, but caused a significant increase in the number of root buds present (Table 6.1; Fig. 6.1D,F). The presence of *Cassida* (low or high densities) had no significant effect on any of the other measured parameters of *C. arvense* (Table 6.1).

Table 6.1. Mean values of each *Cirsium arvense* parameter measured for all treatments in the Garden experiment. All dry weights (DW) and the number of root buds were $\log_{10}$ transformed, and the number of aerial and subterranean shoots was square root transformed. Back-transformed means are given in parentheses. The statistical significance of each main effect contrast are presented. None of the six single-degree-of-freedom interaction contrasts were significant for any plant parameter.

Treatments	Shoot base diameter (mm)	Shoot height (cm)	Sq.rt [number of aerial shoots / plant]		Sq.rt [number of subterranean shoots / plant]		$\log_{10}$ [aerial shoot DW g/ plant]		$\log_{10}$ [subterranean shoot DW g/ plant]		$\log_{10}$ [root DW g/ plant]		$\log_{10}$[total DW g/ plant]		$\log_{10}$ [number of root buds /(g DW)]	
No comp., no *Cassida*	4.2	43.8	4.48	(20.1)	4.76	(22.7)	1.871	(74.3)	0.404	(2.54)	1.374	(23.7)	2.007	(101.6)	0.422	(2.6)
No comp., low *Cassida*	3.6	36.0	4.47	(20.0)	5.12	(26.2)	1.636	(43.3)	0.182	(1.52)	1.074	(11.9)	1.759	(57.4)	0.955	(9.0)
No comp., high *Cassida*	4.2	41.4	4.79	(22.9)	5.39	(29.1)	1.698	(49.9)	0.411	(2.58)	1.208	(16.1)	1.838	(68.9)	0.695	(5.0)
Short clipped, no *Cassida*	4.5	42.9	4.31	(18.6)	4.88	(23.8)	1.738	(54.7)	0.307	(2.03)	1.082	(12.1)	1.845	(70.0)	0.756	(5.7)
Short clipped, low *Cassida*	3.8	34.5	4.50	(20.3)	4.57	(20.9)	1.601	(39.9)	0.307	(2.03)	0.969	(9.3)	1.718	(53.2)	1.195	(15.7)
Short clipped, high *Cassida*	4.4	39.9	4.20	(17.6)	4.51	(20.3)	1.634	(43.1)	0.371	(2.35)	0.984	(9.6)	1.743	(55.3)	1.176	(15.0)
Long clipped, no *Cassida*	4.0	36.6	3.71	(13.8)	4.17	(17.4)	1.526	(33.6)	0.381	(2.40)	1.134	(13.6)	1.708	(51.1)	0.770	(5.9)
Long clipped, low *Cassida*	3.4	35.3	3.66	(13.4)	3.92	(15.4)	1.491	(31.0)	0.328	(2.13)	1.058	(11.4)	1.653	(45.0)	0.896	(7.9)
Long clipped, high *Cassida*	4.6	40.5	3.17	(10.0)	3.82	(14.6)	1.513	(32.6)	0.319	(2.08)	0.877	(7.5)	1.630	(42.7)	0.792	(6.2)
Unclipped, no *Cassida*	4.3	44.1	2.23	(5.0)	1.51	(2.3)	1.115	(13.0)	-0.511	(0.31)	0.702	(5.0)	1.281	(19.1)	0.344	(2.2)
Unclipped, low *Cassida*	5.1	57.6	1.76	(3.1)	2.01	(4.0)	1.296	(19.8)	-0.240	(0.58)	0.699	(5.0)	1.406	(25.5)	0.665	(4.6)
Unclipped, high *Cassida*	5.0	54.8	1.16	(1.3)	1.60	(2.6)	1.195	(15.7)	-0.654	(0.22)	0.660	(4.6)	1.315	(20.7)	0.967	(9.3)
LSD (5%)	**1.3**	**14.5**	**1.21**		**1.73**		**0.234**		**0.548**		**0.303**		**0.222**		**0.389**	
Main effect of competition																
No competition	4.0	40.4	4.58	(21.0)	5.09	(25.9)	1.735	(54.3)	0.332	(2.15)	1.219	(16.6)	1.868	(73.8)	0.691	(4.9)
Short clipped	4.2	39.1	4.34	(18.8)	4.65	(21.6)	1.657	(45.4)	0.328	(2.13)	1.012	(10.3)	1.769	(58.7)	1.042	(11.0)
Long clipped	4.0	37.4	3.51	(12.3)	3.97	(15.8)	1.510	(32.4)	0.343	(2.20)	1.023	(10.5)	1.663	(46.0)	0.820	(6.6)
Unclipped	4.8	52.2	1.72	(3.0)	1.71	(2.9)	1.202	(15.9)	-0.468	(0.34)	0.687	(4.9)	1.334	(21.6)	0.658	(4.5)
LSD (5%)	**0.7**	**8.3**	**0.70**		**1.00**		**0.135**		**0.317**		**0.175**		**0.128**		**0.225**	
Significance of contrasts																
Present vs. absent	ns	ns	***		***		***		*		***		***		ns	
Clipped vs. unclipped	*	***	***		***		***		***		***		***		**	
Short vs. Long clipped	ns	ns	*		ns		*		ns		ns		ns		ns	
Main effect of herbivory																
No *Cassida*	4.3	41.8	3.68	(13.5)	3.83	(14.7)	1.562	(36.5)	0.146	(1.40)	1.073	(11.8)	1.710	(51.3)	0.573	(3.7)
Low *Cassida*	4.0	40.8	3.60	(13.0)	3.91	(15.3)	1.506	(32.1)	0.144	(1.39)	0.950	(8.9)	1.634	(43.1)	0.928	(8.5)
High *Cassida*	4.6	44.1	3.33	(11.1)	3.83	(14.7)	1.510	(32.4)	0.112	(1.29)	0.932	(8.6)	1.632	(42.9)	0.908	(8.1)
LSD (5%)	**0.6**	**7.2**	**0.6**		**0.86**		**0.117**		**0.274**		**0.152**		**0.111**		**0.195**	
Significance of contrasts																
Present vs. absent	ns	ns	ns		ns		ns		ns		*		ns		***	
High vs. low	ns	ns	ns		ns		ns		ns		ns		ns		ns	

*P<0.05; **P<0.01; ***P<0.001; ns = not significant.

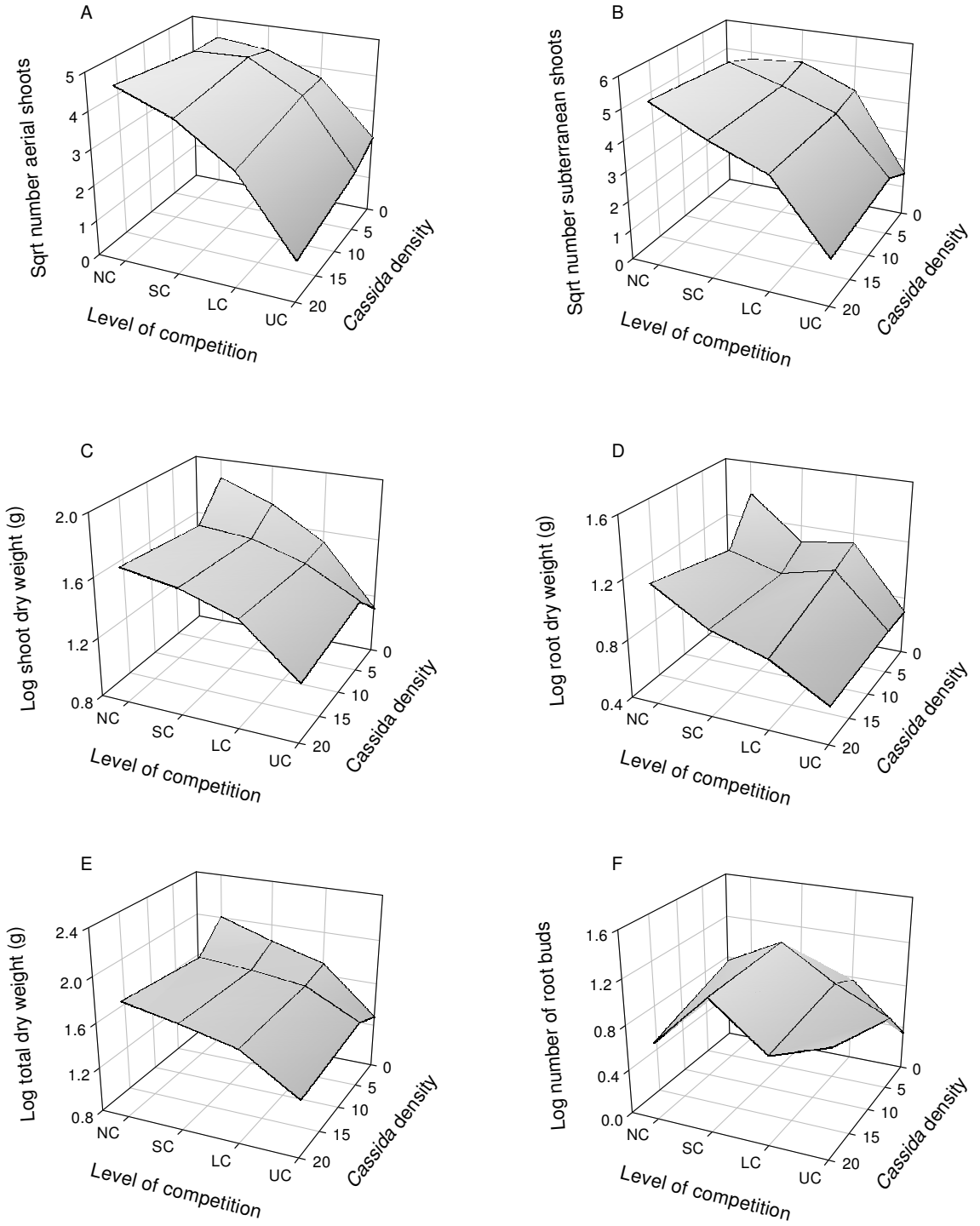

Figure 6.1. Mean *Cirsium arvense* aerial shoot number (A), subterranean shoot number (B), aerial shoot dry weight(C), root dry weight (D), total dry weight (E) and number of root buds (F) in response to increasing levels of plant competition and herbivory by *Cassida rubiginosa* in the Garden experiment. Plant competition levels: NC=Control; SC=Short clipped; LC=Long clipped; UC=Unclipped. Herbivory levels were: no *Cassida*; low *Cassida*=5 larvae per plant; high *Cassida*=20 larvae per plant.

6.3.2 Field experiment

One week after release of larvae (4 June), 46.6% of larvae remained on the shoots, and two weeks after the second release (18 June), 66.8% of larvae remained on the shoots. The growth of *C. arvense* shoots was apparently reduced by *Cassida* compared to the control, but none of the differences were significant at any of the census dates (Fig. 6.2). The proportion of shoots that transitioned from the vegetative to reproductive growth stage did not differ between the control and *Cassida* treatments (deviance ratio = 0.90; P=0.373). This result did not change when using initial shoot height (deviance ratio=0.95) or initial shoot volume (deviance ratio= 1.17) as covariates. The proportion of shoots transitioning from the vegetative to the reproductive stage increased with greater initial shoot height (deviance ratio =35.6; P<0.001), or volume (deviance ratio =64.9; P<0.001), regardless of treatment (Fig. 6.3).

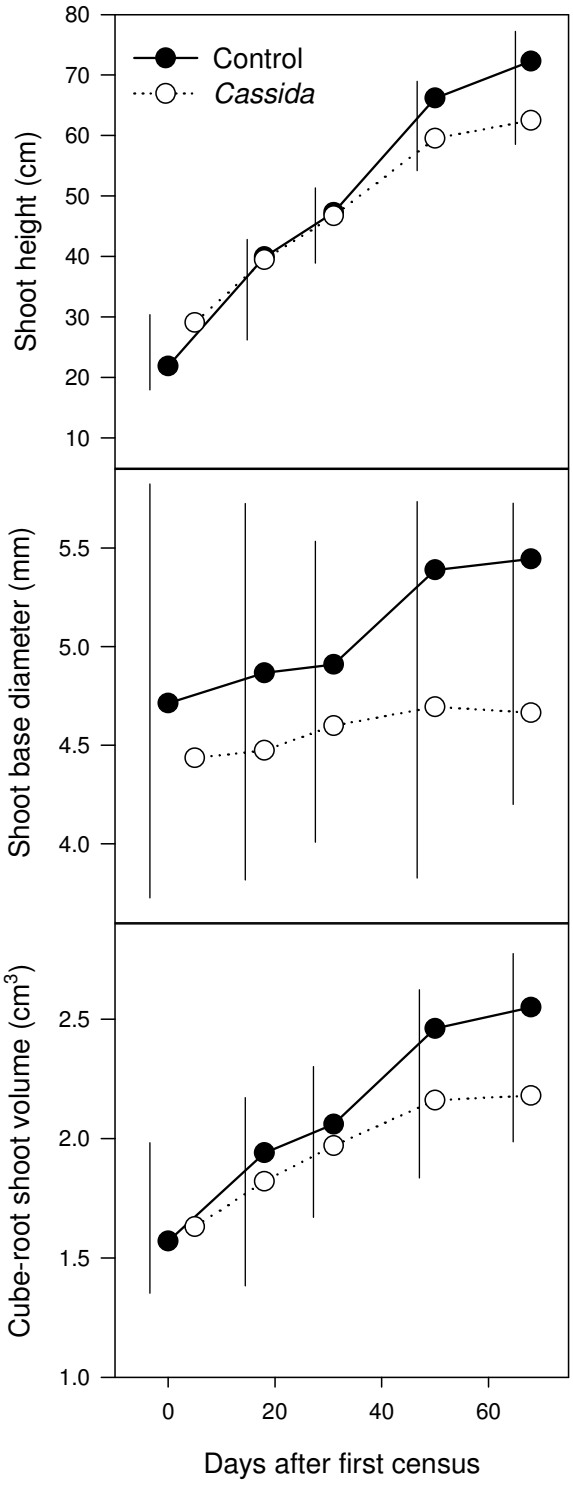

Figure 6.2. Comparisons of mean shoot height (top), mean shoot base diameter (middle), and mean shoot volume (bottom) of *Cirsium arvense* between control and inundation with larvae of *Cassida rubiginosa* in the Field experiment. Vertical lines preceding the data points represent the LSD (5%) value for comparison between the two means.

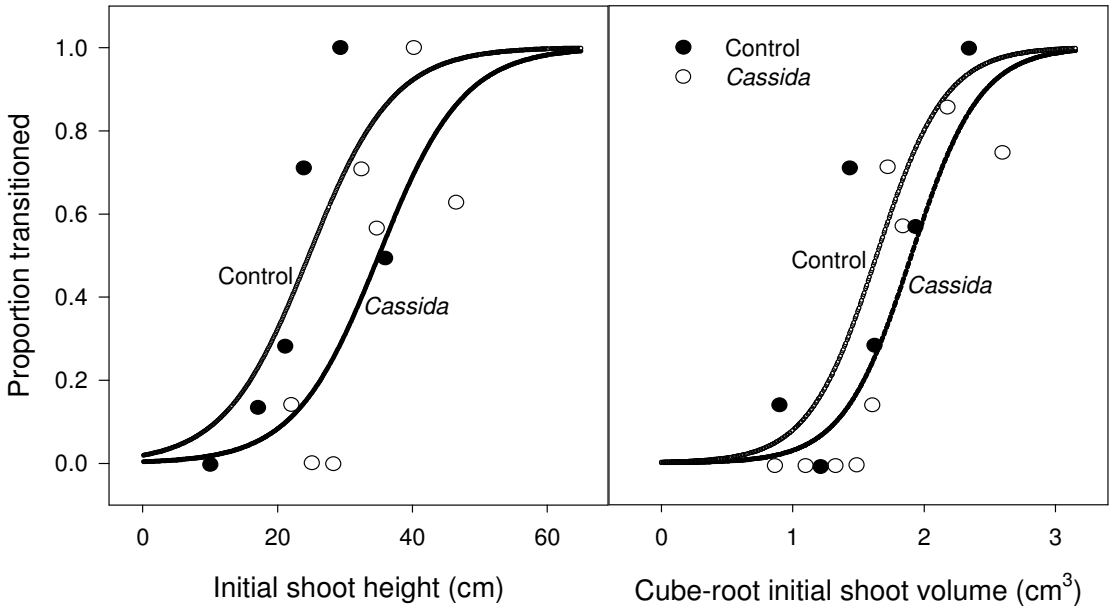

Figure 6.3. Comparison between control and high density release of *Cassida rubiginosa* larvae on the proportion of *Cirsium arvense* shoots that transitioned from the vegetative to the reproductive growth stage when initial shoot height (left) or volume (right) were used as covariates in the Field experiment. Observed data means are shown as triangles. Observed values are the mean proportion of shoots that transitioned to the reproductive stage, where shoots were sorted according to increasing initial height or volume at the time of the first release of *Cassida* larvae, then arranged in successive groups of 6-8 shoots.

6.4 DISCUSSION

6.4.1 Garden experiment

Both interspecific plant competition and *Cassida* feeding had significant impacts on the performance of *C. arvense* in the garden experiment. Competition from the pasture grass and clover was the dominant factor responsible for reducing the performance of *C. arvense*, which is typical of most studies that have investigated the relative impacts of herbivory and interspecific competition (Rees & Brown 1992; Sheppard 1996; Edwards *et al.* 2000; Hinz & Schroeder 2003). In accordance with our first hypothesis, the simulated selective grazing significantly reduced the competitive ability of the perennial ryegrass/white clover mixture, causing *C. arvense* performance to be increased in all response variables except mean shoot height and base diameter, compared to the unclipped treatments. Short vs. long clipping only affected the number and dry weight of aerial shoots of *C. arvense*, which were significantly reduced in the long clipped treatments, as expected. This suggests that the longer sward lengths left by cattle grazing could reduce shoot numbers of *C. arvense* compared to shorter grazing typical of sheep;

however many aspects of grazing other than feeding mode can also be important for management of this weed (De Bruijin & Bork 2006). For instance under rotational grazing livestock may also consume *C. arvense* shoots (Hartley *et al.* 1984).

With regard to our second hypothesis, that *Cassida* would reduce the performance of *C. arvense*, there was only weak support. Of the *C. arvense* response variables measured, only root dry weight was significantly reduced by the presence of *Cassida*, whether low or high densities. Thus, our hypothesis that the affect of *Cassida* on the performance of *C. arvense* would increase with higher larval densities was also not supported. Similarly, in a potted plant trial Friedli & Bacher (2001) found the impact of the oligophagous stem-mining weevil, *Ceratapion onopordi* Kirby on *C. arvense* to be negligible, compared to the impact of competition from grasses. In this case the direct effect of the weevil feeding was thought to be minimal, although a synergistic outcome was reported due an indirect effect of the weevil vectoring the rust pathogen, *Puccinia punctiformis* (Str.) Röhl. (Friedli & Bacher 2001; but see Cripps *et al.* 2009).

Contrary to our third hypothesis, the combination of *Cassida* and competition was not additive for any plant parameter, except for root dry weight, which showed a weak additive response. Similar to our study, a potted plant study using native North American grasses in combination with the monophagous stem-miner, *Ceutorhynchus litura* F., showed an additive effect of competition and herbivory on the reduction of root biomass, but no effect on shoot biomass, of *C. arvense* (Ferrero-Serrano *et al.* 2008). Reducing shoot biomass by mowing has been shown to cause a reduction in root biomass, and number of root buds, which are the overwintering propagules from which new shoots arise the following season (Donald 1993; Bourdôt *et al.* 1998). Thus, it is assumed that if *Cassida* contributed to reducing the overwintering root biomass as a result of foliar herbivory, it would cause population declines in this weed. However, the reduction in root biomass caused by *Cassida* was accompanied by an increase in the number of root buds, which could negate the feeding impact of this biocontrol agent.

The result of significantly more root buds where *Cassida* was present is curious, and we can only speculate what the reason may be. Although this result is contrary to our expectations, it is not necessarily an anomaly, since compensatory effects of plants due to herbivory are well known (Whitham *et al.* 1991; Trumble *et al.* 1993). There are many possible physiological mechanisms of compensation including increased photosynthetic rate in undamaged parts, reallocation of nutrients, and mobilization of assimilates from storage tissues (Whitham *et al.* 1991). There are several examples of plants that reallocate,

or increase uptake, of nutrients to their roots following foliar herbivory (Schwachtje & Baldwin 2008). In fact, Hein & Wilson (2004) showed significantly elevated concentrations of glucose and fructose in the roots of *C. arvense* by the end of the growing season in plants that experienced herbivory from the specialist stem-mining weevil, *C. litura*. It has been hypothesized that perennial plants might store nutrients in unattacked "safe sites" that could allow for future regrowth when herbivory is decreased (Schwachtje & Baldwin 2008). Thus, it is possible that *C. arvense* can compensate for *Cassida* herbivory by investing more resources in bud development that could enhance the future survival of the plant. Previous studies investigating the effect of defoliation on root bud numbers did not examine the effects of specialist herbivores (e.g. Donald 1993; Bourdôt *et al.* 1998); and studies investigating defoliation by specialist herbivores did not measure the number of root buds (e.g. Ang *et al.* 1995; Bacher & Schwab 2000). Therefore previous studies may have overlooked an important aspect of compensation. However, the timing, duration, and intensity of herbivory and the resources available in the given environment could all affect the plant's ability to compensate for herbivory by this beetle.

6.4.2 Field experiment

The field release of *Cassida* larvae simulated a realistic high density of this beetle in Europe, but contrary to our fourth hypothesis, did not cause a significant reduction in shoot size or development of *C. arvense*. This experiment was carried out in a natural population of *C. arvense* in a highly competitive situation (i.e. ungrazed pasture). By contrast, field studies conducted by other researchers have shown significant impacts of *Cassida* larvae on the performance of *C. arvense* in the presence of high levels of interspecific competition (Ang *et al.* 1994; 1995; Bacher & Schwab 2000). For example, in North America, Ang *et al.* (1994; 1995) investigated the impact of *Cassida* combined with competition from a mixture of the grass, *Festuca arundinacea* Schreb., and the legume *Coronilla varia* L. on the performance of *C. arvense*. They found that herbivory by *Cassida* had a significant impact on *C. arvense*, greatly reducing its competitive ability against the forage plants. In Europe, Bacher & Schwab (2000) found that *Cassida* reduced the primary shoot height of *C. arvense*, but only competition reduced the above and below-ground biomass of *C. arvense*. However, their studies differed in several aspects to ours. The experiments conducted by Ang *et al.* (1994; 1995) and Bacher & Schwab (2000) used field plots that were prepared by tillage and then planted root cuttings of *C.*

arvense and sowed seeds of the desired plants for competition. Furthermore, the intensity and duration of herbivory by *Cassida* was greater in their experiments since Ang *et al.* (1994; 1995) used field cages to protect the beetle larvae, and Bacher & Schwab (2000) added beetle larvae twice per week in order to maintain the desired larval density. In contrast to these studies, our field release experiment was carried out in an established population of *C. arvense*, which presumably had a more extensive root system, enabling the plant to more readily compensate for herbivory. Plus, our study plants were not protected by cages, and larvae were added only twice during the entire duration of the experiment. Mortality of the *Cassida* larvae was high in the field release experiment, with approximately half of the larvae missing one week after the initial release, and then approximately one third missing two weeks after the second release. This in not surprising since mortality of *Cassida* larvae due to predation and parasitism is known to be high in the native range of Europe (Bacher & Luder 2005), and also in the introduced range of North America (Ward & Pienkowski 1978; Tipping 1993).

These methodological differences probably account for the non-significant impact of *Cassida* in our study in contrast to other studies. However, the more natural conditions of our experiment are likely a better simulation of reality where predator pressure is high. Furthermore, experiments with artifically high levels of herbivory, or studies conducted on young establishing plants that show significant impacts of biocontrol agents may be misleading. This may also shed light on why biological control of *C. arvense*, at least in North America, has been unsuccessful (McClay *et al.* 2002), even though controlled experiments predict impacts of biocontrol agents. The results of our study highlight the need for well established plants to be included in herbivore impact experiments.

Release from the regulating influence of predators and parasitoids is often thought to facilitate "outbreak" densities of biocontrol agents in their introduced ranges, which are necessary for successful biological control of weeds (Price 1987; Gassmann 1996; Denoth & Myers 2005). In the native range, the simulated high density of *Cassida* inflicted minimal or no impact on *C. arvense*, in both the Garden and Field experiments. Therefore, the success of *Cassida* in NZ may depend on its ability to sustain outbreak population densities, which are unachievable in its native range. Although *Cassida* experiences high rates of predation and parasitism in Europe and North America, there is some reason to speculate that this may not be the case in the novel biotic environment of NZ. In NZ, there are no native Cassidine beetles, and an overall low diversity of the Chrysomelidae (Klimaszewski & Watt 1997), which may correspond with diminished

natural enemy pressure on this group of insect herbivores. Thus, it is plausible that relaxed natural enemy pressure in NZ will be conducive for outbreak populations of *Cassida* that can have an impact on *C. arvense*, and other thistles. Future evaluation of the effectiveness of *Cassida* as a biocontrol agent in NZ should include an assessment of the natural enemy pressure on this herbivore.

Chapter 7

General Discussion

The seminal work of C. S. Elton (1958) drew considerable attention to some of the devastating effects of invasive species. The detrimental impacts of introduced invasive weeds are well documented, but the underlying causal mechanisms by which plants invade new ecosystems are often not well understood. Studying plants in their native and introduced ranges is an approach that can provide valuable insights into mechanisms of plant invasions (Hinz & Schwarzlaender 2004; Hierro *et al.* 2005). Comparative field surveys, such as the one conducted here with *Cirsium arvense* (L.) Scop., can characterise patterns of plant growth, and reveal any extreme differences between ranges. This is important since it is often assumed that introduced plants grow more vigorously in their exotic range, although this perceived increased vigour is seldom quantified.

7.1 Implications of the field surveys

Contrary to expectations the surveys conducted here indicated that the growth of *C. arvense* was generally not different between its native (Europe) and introduced (NZ) ranges (Chapter 3). Incorporating climatic covariates into the analyses revealed that higher precipitation was more favourable for growth of *C. arvense* in NZ. Adjusting for precipitation and altitude showed that if these factors were equal, *C. arvense* growth may be significantly less in the NZ North Island compared to Europe. Although covariate analysis is a useful tool, in this case it has to be interpreted with some caution. It is easy to envision a scenario in which different covariates could cause a different outcome. For instance, if we were able to adjust the means of the *C. arvense* variables for something like soil nutrients, we might have found an opposite pattern, with more favourable conditions in the native range explaining the similar growth patterns. Thus, covariates can offer some insights into the reasons for differences or similarities in growth, but in the end only support speculation about the mechanisms. In essence, the surveys are conducted in order to collect quantitative data to assess the commonly perceived notion that introduced invasive plants are more vigorous in their introduced range, regardless of the reason.

Another benefit of comparative field surveys is acquiring more knowledge about the plant and its associated natural enemies. An interesting find during the NZ field

surveys was the amount of attack by *Rhynocyllus conicus* (Fröl.) in the capitula of *C. arvense* in the NZ North Island. This weevil was released for control of *Carduus* thistles in NZ in 1973 (Julien & Griffiths 1998), and host specificity tests indicated that *C. arvense* was among the host plants of this weevil (Zwölfer & Harris 1984), but the extent to which *C. arvense* was attacked in NZ was previously unknown. Furthermore, its conspicuous absence in the Southland surveys which was also reported by other authors (Fenner & Lee 2001) is curious. The presence or absence of *R. conicus* does not likely account for any differences in plant performance since recruitment from seedlings is rare in established pasture populations of *C. arvense* (Bourdôt *et al.* 2006b). However, the presence of capitula feeders such as *R. conicus* could be important in highly disturbed habitats such as arable fields, since disturbed microsites are known to be important for seedling recruitment (Edwards *et al.* 2000).

7.2 The presence of *Puccinia punctiformis* in Europe and New Zealand: Implcations

The presence and distribution of the specialised rust pathogen, *Puccinia punctiformis* (Str.) Röhl. in NZ is interesting to consider. Cunningham (1927) reported herbarium records of *C. arvense* plants in NZ infected with the rust pathogen dating as early as 1881. Considering that the first record of *C. arvense* in NZ is 1878 it seems unlikely that the plant and pathogen were separated for any considerable length of time. Naturally the question arises as to how the fungus arrived in NZ. It is probably impossible to say for sure, but transoceanic dispersal of *Puccinia* pathogens have been reported for cereal crops (Nagarajan & Singh 1990), and thus long-distance wind dispersal cannot be discounted.

The field surveys confirmed that the specialised rust pathogen, *P. punctiformis*, is wide spread across NZ, although the proportion of shoots infected is generally less than 5% (Chapter 4). The use of the rust pathogen for biological control has been of interest for a long time (Cockayne, 1914; 1915), but has been hampered by an incomplete understanding of the infection process (Frantzen 1994). Within the last decade, studies by a European research group have suggested that a stem-mining weevil (*Ceratapion onopordi* Kirby) can promote rust infection (Friedli & Bacher, 2001; Wandeler & Bacher, 2006; Wandeler *et al.* 2008). An expected, but important piece of information gained during the field surveys, was the complete void of stem-miners inside the shoots of *C. arvense* in NZ, compared to the native range where stem mining was relatively common. Since stem-miners were absent on *C. arvense* in NZ, it was presumed that the release of

C. onopordi might increase the effectiveness of the rust for biological control of this thistle. Interestingly, the field surveys showed that the incidence of rust disease was similarly low in both ranges, with and without stem-miners, respectively. Therefore the idea that stem miners are important vectors of this rust pathogen is doubtful, but the effectiveness of *C. onopordi* and the possibility of increased rust disease will be important factors to monitor following the establishment of this weevil in NZ.

An avenue of research with *P. punctiformis* that has not been fully explored is the possibility of a gene-for-gene type of interaction, as is well known for *Puccinia* diseases of cereal crops (Flor 1971). Two studies provide some limited evidence that this could be the case (Turner *et al.*, 1981; Frantzen & Van der Zweerde, 1994), and if so it could aid greatly in the use of this pathogen as a biological control agent, as in the case of rush skeleton weed, *Chondrilla juncea* (Espiau *et al.* 1998). Although this could be an important mechanism, the genetics of host plant susceptibility/resistance are likely not the only mechanisms by which *C. arvense* escapes infection from this specialised pathogen. Physiological mechanisms are also likely important in escaping disease from this pathogen. For instance, it is common to observe adjacent shoots connected by the same piece of root (i.e. genetically identical), of which one is has rust disease and the other does not. In related work, plant and rust collections were also made during the NZ field surveys in an attempt to test if *C. arvense* ecotypes in NZ exhibited variation for resistance to *P. punctiformis*. Foliar applications of spore suspensions were applied to potted *C. arvense* plants in the glasshouse, but no infection was achieved. This again highlights the difficulty in achieving infection and successful control using this pathogen. Thus, the mechanisms by which *C. arvense* escapes infection by the rust pathogen are unclear, and research that could shed light on this would greatly aid biological control efforts.

7.3 Implications and limitations of the natural enemy exclosure experiment

Although field surveys can reveal patterns of similar or different growth between the native and introduced ranges, they do not offer any mechanistic explanations for the measured growth patterns. The aim of the natural enemy exclosure experiments was to more specifically test the ERH (Chapter 5). The results of this study indicated that insect herbivores caused a reduction in the proportion of shoots reaching the reproductive growth stage. There was also evidence that natural enemies caused reduced population

growth, although there was no consistent trend in whether this was caused by insect herbivores, or pathogens.

A result of the natural enemy exclosure experiments that is somewhat difficult to explain is the reduced growth of *C. arvense* shoots that were treated with fungicide at the field site in St. Ursanne and Greenpark. One of the fungicides used (Tebuconazole) belongs to the triazole class of fungicides, which are known to have inhibitory growth effects (Child *et al.* 1993). Thus, it is possible that the reduced growth was due to a phytotoxic effect of the fungicide application. However, at the Lincoln field site, and in the potted plant experiment, there were no significant effects of the fungicide treatment. If the reduced growth was indeed due to a phytotoxic effect of the fungicide we would expect it to be consistent, and therefore the inconsistency in this negative effect might suggest that *C. arvense* growth was not affected by the treatment. Another possible explanation is that the fungicide treatment may have killed beneficial mutualistic fungi associated with *C. arvense*. This is somewhat speculative since beneficial fungal associations with *C. arvense* are unknown, but probably have not been looked for.

A limitation of this study was that only one field site was used in the native range of Europe. In fact a second field site was initially established in a fallow field in southern Germany, but unfortunately it was destroyed when the field was plowed. It is possible that conditions at the field site could have affected the plants ability to compensate for herbivory. For instance the effects of natural enemies may only have been evident due to resource limitation. Another treatment that would have been valuable to also include in this experiment is grazing. *C. arvense* is a weed in pastures that are typically selectively grazed by livestock. The selective grazing of the livestock can alter the competitive balance between the desirable forage species, and the unwanted thistles. Thus, the different competitive situations with and without grazing could have revealed differences in the influence of natural enemies on *C. arvense*. It is conceivable that the presence of high interspecific plant competition may have suppressed the affects of natural enemies that would have been more pronounced in a grazed or low competitive situation. Conversely, it is also possible that the influence of natural enemies was only detected due to the presence of high competition, as suggested by the impact experiment conducted with *Cassida rubiginosa* Müller. The natural enemy exclosure experiment indicates that natural enemies are capable of regulating *C. arvense*, although this may only be true in certain conditions. The question of whether or not natural enemies are able to regulate plant populations is still a current debate in ecology today (Maron & Crone 2006).

Although new evidence continues to emerge both supporting and negating the regulating influence of herbivores on plant populations, in essence the argument can still be aptly summarized in the words of Harper (1977, p. 510): "the argument about whether plants are regulated by herbivores boils down to a question of how often."

7.4 Implications for biological control of *Cirsium arvense*

Precisely how much natural enemy pressure was present at the Europe site was not clear, but certainly it would vary from one site to the next. How much natural enemy pressure is needed to reduce population growth of the plant is also uncertain. The experiments carried out with *C. rubiginosa* indicate that even with realistically high densities of this natural enemy in Europe, the effect on *C. arvense* was generally not significant (Chapter 6). Therefore, it seems that very high densities of biocontrol agents would be needed to have a significant impact on this weed.

Crawley (1989) noted *C. arvense* as one of the most repeated failures in biological control of weeds. The present research conducted in the native range of the plant offers limited support for the idea that biological control agents can have an impact on *C. arvense*. In all chapters no strong conclusions could be made concerning the effectiveness of the newly released biological control agents in NZ. This is primarily because it is difficult to predict how a biocontrol agent will respond to a novel environment such as NZ. Since these biocontrol agents are released from their own natural enemies (predators and parasitoids) it is possible that they may be able to reach higher population densities in NZ compared to their native range, and thereby have an impact on the weed. In NZ there are no native tortoise beetles (subfamily Cassidinae) and low Chrysomelid beetle diversity. Therefore there is some reason to speculate that that *C. rubiginosa* may find some enemy-free space in NZ and be able to reach the high densities necessary to have an impact on thistle plants.

7.5 Future research

Future studies testing the influence of natural enemies on *C. arvense* would benefit by varying the environmental (i.e. resource availability) and compeititve interactions that would alter the plant's ability to compensate for herbivory. Also, in order to assess the impact of natural enemies on *C. arvense*, studies should follow generations of the plant, rather than only life-time fitness or performance of the plant. For instance the single-season study conducted here with *C. rubiginosa* indicated that *C. arvense* compensated

for foliar herbivory by increasing production of root buds. However, whether or not this translates into increased shoot density the following season is uncertain, and would require multiple generation monitoring. Follow-up studies with *C. onopordi* and *C. rubiginosa* in NZ will be important to assess the impacts of these agents on *C. arvense* (and other thistles), and to assess the rates of predation and parasitism that these agents might experience in NZ. Furthermore, the potential for *C. onopordi* to increase the incidence of rust disease would also be valuable to follow up on in NZ. Future studies testing the enemy release hypothesis would benefit by using invasive weeds with different life histories (e.g. annual vs perennial, or clonal vs. non-clonal), and by using environmental weeds, rather than agricultural weeds, which occur in more artificial habitats, and might not respond the same to enemy release.

7.6 Conclusions

The data from this thesis indicate that endophagous herbivore attack in the stems and capitula of *C. arvense* is significantly greater in the native range, as predicted by the ERH. Interestingly, the survey data also showed that the proportion of shoots attacked by the specialised rust pathogen, *P. punctiformis*, was not significantly different between ranges. This data questioned the idea that stem-mining insects are important vectors of this rust pathogen, and has further highlighted the value of conducting biogeographical surveys. Although natural enemy pressure is greater in the native range, performance of *C. arvense* is not significantly different in its native range, compared to its introduced range of NZ; or differences can be explained by simple climatic factors such as precipitation. This survey data showed that the commonly perceived notion that *C. arvense* is more vigorous in NZ compared to its native range is incorrect, and does not support the prediction of the ERH. However, limitations of the field surveys are also evident, and therefore permanent field plots were also established in each range in order to more specifically test the ERH. From the permanent field plots where natural enemies were exluded and compared with control plots (ambient natural enemy pressure) there was evidence that natural enemies had an effect on the population growth and shoot development of *C. arvense*, providing support for the ERH. Which natural enemies were causing these effects is uncertain, but the fact that natural enemies can influence *C. arvense* offers some support for biological control of this weed. The biological control agent, *C. rubiginosa*, can also have an impact on *C. arvense*, but possibly only at high densities, and when the plant is weakend by strong

interspecific competition. The data from this thesis has also shown that valuable insights into invasion mechanisms of introduced weeds can be gained by conducting biogeographical studies in the native and introduced ranges.

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Appendix 1

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SHORT COMMUNICATION

Does transmisson of the rust pathogen, *Puccinia punctiformis*, require stem mining vectors?

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Recent research in Europe has suggested that stem mining insects may be important for vectoring the pathogen *Puccinia punctiformis*, and largely responsible for its systemic infection in the weed, *Cirsium arvense*. However, here we present comparative survey data showing that the level of systemic disease is the same in Europe and New Zealand, with and without stem miners, respectively, casting doubt on the idea that these insects are necessary for transmission of the fungus.

Keywords: *Apion onopordi*; *Ceratapion onopordi*; Canada thistle; Californian thistle; pathogen vector

Cirsium arvense (L.) Scop. (Californian, Canada, or creeping thistle) is one of the worst weeds in New Zealand (NZ) arable and pastoral production systems (Bourdôt and Kelly 1986; Bourdôt et al. 2007). It is a species of Eurasian origin that was accidentally introduced to NZ approximately 130 years ago. Not long after its establishment, the highly specialised rust fungus, *Puccinia punctiformis* (Str.) Röhl., was also noted to be widespread on *C. arvense* (Cunningham 1927). Its potential as a biocontrol agent in NZ did not go unnoticed, but was hampered by an incomplete understanding of the factors affecting the infection process and disease development (Cockayne 1914, 1915). This pathogen is an attractive biocontrol agent because the systemic disease it causes has severe detrimental effects on the plant, often killing shoots before they flower (Watson and Keogh 1980; Thomas, Tworkoski, French, and Leather 1994). However, the infection process remains obscure, in particular, how systemic infection is initiated and how subsequent disease arises. The current understanding is that systemic disease arises from adventitious shoot buds contacting basidiospores from germinated teliospores in the soil (Van den Ende, Frantzen, and Timmers 1987; French and Lightfield 1990; Frantzen 1994). However, studies attempting to increase systemic disease by augmenting spore levels have met with limited success. Kluth, Kruess, and Tscharntke (2003) found that by actively

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spreading urediniospores (that produce urediniosori that morph into teliosori with teliospore production) onto plants, the number of systemically diseased shoots could be increased the following season; but this did not result in a significant reduction in the number of flowering shoots. Furthermore, experiments applying spore suspensions to the soil did not result in an increase of systemically diseased shoots in field trials (Frantzen and Scheepens 1993).

More recently, there has been increasing evidence suggesting that stem mining insects act as vectors of the pathogen and are largely responsible for systemic infection and disease (Friedli and Bacher 2001a; Wandeler and Bacher 2006; Wandeler, Nentwig, and Bacher 2008). This recent research has focused on the oligophagous stem mining weevil, *Ceratapion* (=*Apion*) *onopordi* Kirby. The proposed mechanism is that the female weevil carries urediniospores and inoculates shoots via ovipositional punctures in the stem base (Friedli and Bacher 2001a; Wandeler and Bacher 2006). This was successfully demonstrated in a natural field population where systemic disease was increased in the vicinity of shoots experimentally infested with spore-covered weevils (Wandeler et al. 2008). Several specialised stem mining insects exist on *C. arvense* in the plant's native range (Freese 1994; Zwölfer 1965) that could be capable of vectoring the pathogen in the manner proposed by Friedli and Bacher (2001a). The research indicating that *C. onopordi* is an important vector of the rust pathogen was used to support its approval for field release in NZ. It was assumed that the weevil would work synergistically with the rust pathogen to enhance biological control of *C. arvense* (Friedli and Bacher 2001a,b). Previously the level of rust and the amount of systemic vs. localised disease in NZ was unknown. Here we present comparative survey data on levels of rust in the native (Europe) and introduced (NZ) ranges of the thistle, and question the ecological importance of stem miners for vectoring *P. punctiformis*.

In summer of 2007, surveys of *Cirsium arvense* were conducted in 13 populations in New Zealand, and 12 populations in Europe (Table 1). Surveys were carried out in both the North and South Islands of NZ; and in Germany, France, Switzerland, Hungary and Croatia, in Europe (Table 1). The surveys were carried out during the flowering to fruiting period of the plant. In New Zealand this was from late January to mid February, and in Europe from June to early August. At each field site the land area occupied by the *C. arvense* population was estimated. A population was defined as a continuous patch without separation between adjacent shoots of more than 50 m (Lalonde and Roitberg 1994). A transect of up to 40 m was randomly placed within each population (patch). Quadrats (1 m^2) were systematically placed at 2-m intervals along the transect up to a maximum of 20 quadrats. In each quadrat the number of healthy and rusted *C. arvense* shoots was counted and totalled for each population. Rusted shoots were classified as having either systemic or localised disease. In addition, the shoots were harvested from every second quadrat (maximum 10 quadrats) and the stems of three healthy shoots were dissected (maximum 30 shoots) and examined for stem mining insects that could potentially vector the rust pathogen.

The proportion of diseased shoots was compared between Europe and New Zealand using a generalized linear model (GLM) with a logit-link function, allowing for over-dispersion and assuming a binomial distribution (with the binomial total being the sum of the total number of shoots in each population). This analysis was also carried out including only those populations where rust was detected.

Table 1. *Cirsium arvense* populations surveyed in Europe and New Zealand, 2007.

Population code	Site name	Country/ Region	Coordinates[1]		Patch size (m^2)
European populations					
CH1	Müntschemier	Switzerland	N 46° 58.906′	E 007° 08.443′	82
D1	Grissheim	Germany	N 47° 52.784′	E 007° 35.247′	420
D2	Müllheim	Germany	N 47° 48.898′	E 007° 35.377′	1600
F1	Ligsdorf	France	N 47° 26.398′	E 007° 18.103′	1500
H1	Sopron	Hungary	N 47° 42.428′	E 016° 34.532′	1500
H2	Balf	Hungary	N 47° 39.996′	E 016° 40.085′	300
H3	Sarród	Hungary	N 47° 39.349′	E 016° 51.744′	15,000
H4	Babót	Hungary	N 47° 33.980′	E 017° 04.796′	5000
H5	Celldömölk	Hungary	N 47° 15.953′	E 017° 09.669′	180
H6	Rédics	Hungary	N 46° 49.126′	E 016° 34.739′	7500
HR1	Legrad	Croatia	N 46° 12.608′	E 016° 44.040′	2000
HR2	Đurdenovac	Croatia	N 45° 31.974′	E 018° 01.300′	600
New Zealand populations					
BP1	Waihi 1	Bay of Plenty	S 37° 26.952′	E 175° 51.557′	3000
BP2	Katikati 1	Bay of Plenty	S 37° 33.365′	E 175° 54.397′	600
BP3	Katikati 2	Bay of Plenty			180
W1	Pukeatua 1	Waikato	S 38° 03.640′	E 175° 32.667′	2250
W2	Pukeatua 2	Waikato			1400
AK1	Huntly 1	South Auckland	S 37° 35.065′	E 175° 06.852′	260
AK2	Huntly 2	South Auckland			192
O1	Clinton	South Otago	S 46° 09.438′	E 169° 32.975′	7500
SL1	Otautau	Southland	S 46° 06.972′	E 168° 01.748′	800
SL2	Papatotara 1	Southland	S 46° 09.395′	E 167° 36.358′	9000
SL3	Papatotara 2	Southland			850
SL4	Fairfax 1	Southland	S 46° 11.538′	E168°02.422′	3000
SL5	Fairfax 2	Southland			1000

[1]Populations without coordinates were within 1 km of the site with the same name.

The proportion of quadrats infected out of the total along the transects in the populations that had rust present was analysed in the same way. The three analyses correspond to examining overall disease levels, and incidence of disease where it was present, at the levels of the population and quadrat. Analyses were conducted using GenStat (Version 10.1).

From the field surveys of *C. arvense* conducted in both the native (Europe) and introduced (NZ) ranges, 7 out of 12 populations contained rust in Europe, and 8 out of 13 populations contained rust in NZ. There was no significant difference between ranges in the mean percentage of diseased shoots (back-transformed means $\pm$SE for EU $=0.78\pm0.35$ and NZ $=0.84\pm0.34$; deviance ratio $=0.02$, $P=0.900$; Figure 1). There was also no significant difference between ranges in the mean percentage of shoots infected when comparing only diseased populations (back-transformed means $\pm$SE for EU $=2.3\pm0.69$ and NZ $=1.7\pm0.47$; deviance ratio $=0.45$;

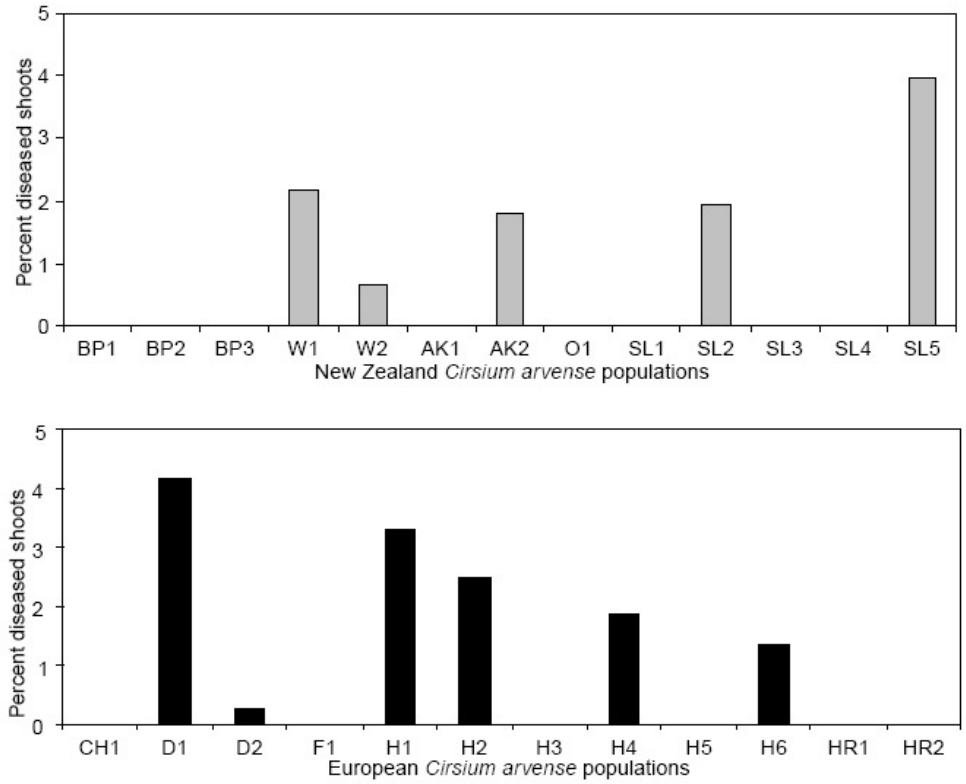

Figure 1. Percent *Cirsium arvense* shoots with rust disease out of the total number of shoots counted for each population. The populations BP1, BP2, SL4 (NZ), and H3 (Europe) had a low levels of rust that was not detected by the survey method.

$P = 0.517$). The percentage of quadrats containing rust along the transect (for diseased populations only) was also not significantly different between ranges (back-transformed means$\pm$SE for EU $= 13.3 \pm 3.0$ and NZ $= 17.0 \pm 3.7$; deviance ratio $= 0.60$; $P = 0.458$). When averaged over all rusted shoots, 97% were classified as 'systemic' in Europe and 88% in NZ, and the small remaining proportions were classified as 'localised' disease. In Europe, 12.4% of the *C. arvense* stems were attacked by various stem mining insects, but no such attack was found in any of the *C. arvense* surveyed in NZ.

Previously the proportion of rusted *C. arvense* shoots, and the amount of systemic vs. localised disease was unknown in NZ. Quantifying the level of rust in NZ was considered important for obtaining baseline data prior to the release of *C. onopordi*, so that the expected increase in damage to the thistle populations could be quantified. The surveys conducted in this study show that there is no difference in the level of rust between the native (Europe) and introduced (NZ) ranges of *C. arvense*. This is true when comparing the proportion of diseased shoots in the population and the number of quadrats containing rust along the population transect. It is also evident that there are no specialised insect herbivores feeding inside the stems of *C. arvense* in NZ. The monophagous stem-mining weevil, *Ceutorhynchus litura* (F.), was released in 1976, but failed to establish anywhere in NZ (Harman, Syrett, Hill, and Jessep 1996). Only two specialised insects are

known on *C. arvense* in NZ: *Lema cyanella* (L.) and *Rhinocyllus conicus* (Frölich). *Lema cyanella* is a monophagous leaf chewing chrysomelid beetle that has been confirmed as established at only one site in NZ (Landcare Research, unpublished data). *Rhinocyllus conicus* is an oligophagous seed feeding weevil that was released for control of *Carduus nutans* L. (its preferred host) in NZ, but has also been found on *C. arvense* (Zwölfer and Harris 1984). This confirms the lack of establishment of biocontrol agents previously released for control of *C. arvense* in NZ noted by other authors (Harman et al. 1996; Julien and Griffiths 1998). In NZ only five generalist insect herbivores have been reported in association with *C. arvense* (Spiller and Wise 1982), and the only insects commonly encountered on *C. arvense* in NZ are pollinators (M. Cripps, unpublished data). In essence, there are no stem mining insects in NZ that could vector the rust pathogen in the manner proposed by Friedli and Bacher (2001a). Since the incidence of rust disease is similar in both ranges, this raises the obvious question: how important are stem miners for vectoring this pathogen?

The idea that stem mining insects may increase systemic infection and disease is not new. In North America, there was speculation that the monophagous stem mining weevil, *Ceutorhynchus litura*, was increasing the level of rust (Peschken and Beecher 1973); however, it was later reported that this could not be substantiated (Peschken and Wilkinson 1981). Research indicating that *C. onopordi* is important for promoting systemic disease of *P. punctiformis* in *C. arvense* (Friedli and Bacher 2001a) contributed to interest in this weevil as a biological control agent in NZ, where the rust is already present. Friedli and Bacher (2001a) demonstrated that *C. onopordi* shows a preference for thistle shoots at an early stage of systemic disease when shoots are bearing pycnia and emitting a characteristic floral odour. In Europe, the weevil by itself causes minimal impact on *C. arvense*; however, combined with the rust fungus the impact on *C. arvense* was believed to be synergistic (Friedli and Bacher 2001b).

Bacher and Friedli (2002) suggested that a mutualistic relationship exists between the weevil and the rust. They proposed that the fungus benefits from dispersal by the weevil, and the weevil benefits from increased fitness when developing on rusted shoots (Bacher, Friedli, and Schar 2002). Since the weevil shows a distinct preference for diseased shoots, and healthy thistle shoots are a suboptimal host (Friedli and Bacher 2001a), there is an obvious quandary as to how new healthy shoots are infected. Bacher and Friedli (2002) address this issue and suggest that host finding ability is based on the relative proportion of healthy to diseased shoots in a population. At low frequencies of rust (below 23%) more healthy shoots should be attacked, in theory due to the weevil's poor host finding abilities (Bacher and Friedli, 2002; Moravie, Borer, and Bacher 2006). Interestingly, the model of the dynamics among the weevil, rust and host plant does not consider the presence of the preferred host plant, *Cirsium vulgare* (Savi) Ten., which often co-occurs with *C. arvense* in both Europe and NZ (M. Cripps, personal observation).

The idea of a mutualistic interaction between herbivores and *P. punctiformis* is contrary to most knowledge about insects and biotrophic fungi. In general, *Puccinia* fungi are considered to be dispersed as windblown spores (Agrios 2005), and the interaction between insects and biotrophic fungi such as *Puccinia* is believed to be antagonistic (Hatcher 1995). This is because biotrophic fungi require living host plants for development. Thus, a herbivore that might weaken or accelerate the death of the host would be detrimental to the fungus. However, Friedli and Bacher (2001a)

argue that if the insect spreads the pathogen more effectively, any fitness loss from the minimal impact of the insect will be off-set by the benefits of increased dispersal. The data presented here indicate that the rust fungus is similarly prevalent in Europe and NZ, indicating that a mutualistic relationship between the fungus and stem miners is not obligatory.

A further point of interest is the suggestion that urediniospores are the cause of systemic infection (Wandeler and Bacher 2006; Wandeler et al. 2008). In general, the life cycles of *Puccinia* fungi are fairly well understood (Scott and Charkravorty 1982; Agrios 2005). Dikaryotic urediniospores ($n+n$) develop into teliospores ($2n$) via the process of karyogamy. Germinating teliospores give rise to basidiospores (n), which cause infection, and subsequent production of monokaryotic pycniospores (n) – the characteristic spore stage indicative of systemic disease. Thus, it is unlikely that dikaryotic urediniospores – by some other process – cause systemic infection and diseased shoots bearing monokaryotic pycniospores. Other authors have noted that some teliospores are always present in uredia (van den Ende et al. 1987); and, Wandeler and Bacher (2006) also report that a small proportion of teliospores were present in the inoculum carried by the weevils. Given the unlikelihood of urediniospores causing systemic infection, the teliospore contamination should not be excluded as a probable cause of the systemic disease observed in their studies.

We do not doubt that *C. onopordi* is capable of vectoring the rust, as are other specialised insects (Kluth, Kruess, and Tscharntke 2002). However, given that specialised insects do not exist on *C. arvense* in NZ (except *L. cyanella* on foliage and *R. conicus* in the seedheads) and that the rust is as common in NZ as it is in Europe, it is highly unlikely that insect vectors are necessary for transmission of this pathogen. If there were higher levels of systemic disease in Europe, this might indicate that the weevil is capable of increasing the incidence of systemic infection and disease. However, we have shown that the level of systemic disease is not different between ranges indicating that the weevil may not be necessary as a vector of this pathogen. It should be noted that our survey was based on only 1 year, and incidence of rust can vary from year to year according to environmental conditions (Scott and Charkravorty 1982). Furthermore, our surveys were carried out only once in each population, which would not capture any variation in the amount of rust, or the proportion of systemic vs. localised disease, from early to late season. Therefore, it is possible that the conditions during our survey season were not favourable for rust development in Europe, and that *C. onopordi* may be capable of increasing the level of rust during a season with more favourable environmental conditions. Genetic variation in clonal resistance/susceptibility to the rust pathogen is also reported to exist (Turner, Fay, Sharp, and Sands 1981; Frantzen and Van der Zweerde 1994), and may also influence the level of rust observed in NZ. The data presented here indicate that stem mining vectors are not necessary for transmission of the rust pathogen, which may have implications for efforts to enhance biological control of *C. arvense*. However, the outcome of any interactions among *C. onopordi*, the rust, and *C. arvense* in NZ remains to be determined.

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